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NEW LIGHT ON BLENDING AND MENDELIAN INHERITANCE

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THE question whether blending or intermediate inheritance involves Mendelian principles is of prime interest to every student of genetics. The further question whether Mendelian characters are constant or variable is not less important. New light is shed on both these questions by a recent and valuable investigation by Y. Hoshino, "On the Inheritance of the Flowering Time in Peas and Rice."

The investigation is to be most highly commended for its thoroughness. It has involved the raising of over 30,000 plants extending over a period of 8 years. F_2 and F_3 generations have been raised three times and F_4 twice. The publication contains a careful summary and analysis of the facts observed. Above all it contains *facts* with a minimum of speculation, not facts so marshalled as to prove a particular theory and otherwise useless, but facts which the reader may study from any angle he chooses. I propose to exercise the privilege which our author's commendable method of publication makes possible, of utilizing his facts for testing a slightly different theoretical interpretation from that which he adopts, for I am acquainted with no material equally valuable in relation to the two questions stated above, though I have been engaged for many years in studying these same questions.

Hoshino crossed two varieties of peas which differed in

flowering time by about three weeks. The early variety was white-flowered, the late variety red-flowered. The F_1 hybrids were only a little earlier than the late variety in time of flowering and of course had red flowers, this being a dominant character. The F_2 hybrids varied in time of flowering throughout the range of both parent varieties and all intermediate points, but not beyond the range of the parents. Hoshino divides the F_2 plants into two main groups, an early flowering and a late flowering group, the two being separated naturally by a class of "minimal frequency" usually recognizable. A majority of the plants in the early flowering group had white flowers, a majority in the late flowering group had red flowers. This result shows that early and late flowering have a tendency to segregate from each other as Mendelian allelomorphs and that time of flowering is *coupled* with flower color. This fact of coupling, first observed by Lock, alone would suffice to show that time of flowering is inherited as a Mendelian character. The fact that the F_2 individuals apparently fall into two main groups, not into four or eight, indicates that one important Mendelian factor is concerned, not two or three. But Hoshino found that in F_3 four (not two) groups of relative constancy were obtained and this has led him to suppose that the inheritance of time of flowering depends upon *two* independent Mendelian factors, one of which (A, a) determines late or early flowering, while the second (B, b) supplements or modifies the action of A. He regards A as the *principal* factor, with which alone flower color shows coupling. The action of B is regarded as subsidiary. A characterizes late-flowering plants; a, early-flowering plants. B makes the flowering time later than it would be otherwise; b of course makes it earlier. The four supposedly homozygous sorts which are recognized are of the following formulæ:

aabb, early; aaBB, intermediate early; AAbb, intermediate late; AABB, late. Since red color of flowers is by hypothesis coupled with A, red flowers will predominate in the last two groups, white flowers in the first two

as observed, and the dividing line between them will correspond with Hoshino's class of minimal frequency in F_2. But the plants which Hoshino classified, on the basis of F_3 and F_4 tests, as extracted early (aabb) and extracted late (AABB) are usually not quite so early or late, respectively, as the uncrossed races. Hence he assumes the occurrence of "gametic contamination," and recognizes classes of "would be" or "pseudo"-early and "pseudo"-late. He also notes the occurrence of "qualitative" variation within the groups classed as "constant" early intermediate and "constant" late intermediate. That is one family supposed to be of the same genetic formula as another may throughout its entire range produce plants slightly *earlier* or *later* than those of the other family. This behavior is not ascribed to any difference in genetic formula, but to a slightly different value of the same gene in the two families.

The late race employed was also found to vary in lateness, one "pure line" derived from it being later than another. Crosses made with these two lines are reported separately. No factorial difference is recognized between them. Each is AABB, but one flowers a little later than the other and transmits this property to its descendants. Thus "qualitative" variation of a gene, *i. e.*, variation in its potency, is recognized by Hoshino. Aside from the occurrence of two pure lines in the late race, Hoshino considers "the flowering time quite fixed and unchangeable in the parent varieties," and cites his Tables IV–VI in support of this idea. Table VI is of particular interest in this connection because in this case seeds were planted of the earliest flowering and latest flowering individuals of the same pure line and descended from the *same individual grandparent*. These plantings constitute a test of the existence of genetic variation within the pure line. The progenies of the same grandparent plant (but of different parents) are so obviously alike and so little variable in flowering time that Hoshino has not considered it necessary to calculate their mean flowering time. But if this is done it affords unmistakable evidence that genetic varia-

tion occurs within these "pure lines" (see Table I). For in all but the last two of the thirteen cases tested the earlier parent has the earlier progeny. From long experience in studies of rats with such small differences as are here indicated I have no hesitation in concluding that fluctuating variation of genetic significance is here in evidence.

To recapitulate, as regards genetic variation in flowering time, Hoshino (1) recognizes that gametic contamination results from crossing early and late flowering varieties; (2) recognizes also that variation may occur among the cross-bred families, as well as in different pure lines of the uncrossed races, as regards the "quality," value, or potency of the same gene. (3) Although Hoshino does not refer to the fact, his observations show clearly that genetic variation of a graded or fluctuating sort occurs in at least one of the varieties which he crossed. It is probable that those varieties were as pure as are obtainable, but almost certain that their flowering time fluctuates slightly from genetic causes.

What I want to suggest is that in these several agencies we have a sufficient explanation of the variations observed in Hoshino's F_2, F_3, and F_4 generations, without invoking a two-factor hypothesis, one factor being enough. Hoshino has shown that a three-factor, or multi-factor hypothesis will not fit the facts observed. Will not *one* factor fit them quite as well as two, provided gametic contamination occurs, which he admits? The "pseudo-early" and "pseudo-late" classes Hoshino explains plausibly as due to gametic contamination. Could not the "intermediate early" and "intermediate late" be reasonbly explained as due to *further* contamination? For they intergrade with the pseudo-early and pseudo-late classes, respectively, and also with each other. From Hoshino's carefully controlled results, it is perfectly clear that early and late flowering are allelomorphs, and that segregation of early and late types occurs in F_2 but attended by gametic contamination. It is perfectly clear that the contamination is not uniform in amount. Some-

times little or no contamination is observable; sometimes it is considerable.

TABLE I

COMPARISON OF THE MEANS OF FAMILIES DESCENDED FROM THE SAME PURE-LINE GRANDPARENT, BUT FROM PARENTS OF UNLIKE CHARACTER

Based on Hoshino's Table 6.

Designation of Grandparent	Mean, Progeny of Earlier Parent	Mean, Progeny of Later Parent	Difference
Gp. I.4	59.96	60.11	.15
Gp. XVI.3	59.94	60.65	.71
Gp. XII.8	60.16	60.50	.34
Gp. VIII.3	63.65	63.98	.33
Gp. VIII.4	63.86	64.21	.35
C.12	60.25	60.58	.33
C. 65	59.43	60.05	.62
C. 61	62.91	64.18	1.27
C. 88	64.23	64.56	.33
Gp. XVI.3.5	66.45	66.63	.18
C. 65.27	66.22	67.30	1.08
Gp. VIII.4.23	71.75	71.20	−.55
C. 61.21	72.00	71.25	−.75

Contamination sufficiently great would account for the intermediate early as a *modified* early class and the intermediate late as a *modified* late class. The matter of coupling is unaffected by this hypothesis, since coupling is shown with only *one* factor, Hoshino's factor A. The observed variability and intergradation of the two intermediate classes favors a hypothesis of contamination rather than one of an independent modifying factor.

If we suppose modification due to gametic contamination to occur in half of the gametes formed by F_1 individuals and that this contamination is definite in amount (say equivalent to 5 days) the F_2 expectation would be exactly the same as from a two-factor system such as Hoshino adopts. As a matter of fact neither supposition is exactly correct. If we adopt contamination as a sufficient explanatory hypothesis, we must suppose the amount of contamination to be variable; if we adopt a definite modifying factor, we must suppose the amount of modification to be variable.

To make the matter clear, let us suppose the early race to be stable at 35 days between sprouting and flowering

and the late race to be stable at 55 days. According to Hoshino's hypothesis aabb stands for a 35-day period, and AABB for a 55-day period. If we assign to the assumed modifying factor B a delaying effect of five days, then the class aaBB (early intermediate) will have a value of 40 days, and the other homozygous class AAbb (late intermediate) will have a value of 50 days. Heterozygous classes will be intermediate as follows:

TABLE II

COMPOSITION OF F_2 ON HOSHINO'S TWO-FACTOR HYPOTHESIS, WITH EQUIVALENTS IN DAYS FROM SPROUTING TO FLOWERING

Designation and Expected Frequency	Homozygous	Heterozygous	
		Monohybrid	Dihybrid
1 aabb	35		
2 aaBb		37.5	
1 aaBB	40		
2 Aabb		42.5	
4 AaBb			45
2 AaBB		47.5	
1 AAbb	50		
2 AABb		52.5	
1 AABB	55		

If, however, we replace the modifying factor B in this scheme by a modification in A amounting to 5 days, then we can dispense with B and yet obtain exactly the same classes and in the same numerical proportions and with nearly the same expectations as regards their breeding capacity. Let us assume that a stands for a 35-day period, A for a 55-day period, and that modified a, which we will call a′, stands for a 40-day period, and modified A, which we will call A′, stands for a 50-day period and that all are allelomorphs of each other. Then the F_1 gametes will be $a + a' + A' + A$ and F_2 will contain the classes shown in Table III.

It is evident that both schemes fit the observed facts fairly well. Either one will explain the decreased variability of F_3 as compared with F_2 and the production of several different types of F_3 families differing in the amount of their variability, some of which are relatively constant. But the former scheme will not answer with-

out the further assumption of gametic contamination (which Hoshino makes) and the latter must invoke contamination of different degrees in order to explain the pseudo-early and pseudo-late classes. The former scheme involves two explanatory principles, the latter only one. Other things being equal, the simpler hypothesis is to be preferred.

TABLE III

COMPOSITION OF F_2 ON A ONE-FACTOR HYPOTHESIS WITH CONTAMINATION OF A DEFINITE AMOUNT (5 DAYS) IN HALF THE GAMETES

Designation and Frequency	Homozygous	Heterozygous, All Monohybrid			
		Difference, Conj. Gam., 5	Difference 10	Difference 15	Difference 20
1 aa.....	35				
2 aa'.....		37.5 (35:40)			
1 a'a'.....	40				
2 aA'.....				42.5 (35:50)	
2 aA.....					45 (35:55)
2 a'A'.....			45 (40:50)		
2 a'A.....				47.5 (40:55)	
1 A'A'.....	50				
2 A'A.....		52.5 (50:55)			
1 AA.....	55				

Experiments decisive between the two hypotheses are difficult to devise, but certain tests are possible. On the hypothesis of Hoshino one would not expect to obtain a class splitting into homozygous early intermediate and homozygous late intermediate. On the alternative hypothesis such a class (40–50) should be obtainable. *A "constant" class exactly intermediate between the parent varieties* (say 45) *would be impossible on Hoshino's hypothesis,* unless he is willing to admit an indefinite amount of contamination, which, however, would render the two-factor hypothesis superfluous. On the alternative hypothesis such a constant intermediate class should be obtainable after a sufficient number of inbred generations. In reality Hoshino's observations show that *it is obtained in F_4 and is then more abundant than any other "constant" class.* The largest group of hybrid offspring in Hoshino's experiments and that belonging to the latest inbred generation is his F_4 generation raised in 1914 (see

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2 aA					45 (35:55)
2 a′A′			45 (40:50)		
2 a′A				47.5 (40:55)	
1 A′A′	50				
2 A′A		52.5 (50:55)			
1 AA	55				

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his Tables G–U). This includes 231 families classed by Hoshino as "constant." They are descended from 15 different F_2 individuals ranging in flowering time (in 1912) from 48 to 65 days, this being practically the entire F_2 range. The combined range of the F_3 families raised in 1913 extended from 45 to 71 days. From certain individuals, selected at intervals throughout the ranges of the fifteen F_3 families so as to represent their complete variability seeds were planted which produced the F_4 generation. 421 such F_4 families were reared and studied and of these Hoshino regards 231 as "constant" because of the limited range of variation in flowering time of each. The others are regarded as still heterozygous. The mean flowering time of each of the "constant" families has been calculated by Hoshino and these means have the distribution shown in Table IV (omitting fractions from the class magnitudes which would make them .5 greater than those given in the table, but would not affect their distribution). It should be noted that on account of the peculiar weather conditions of 1914, the flowering time came about 10 days earlier than in the two previous years, the range of the F_4 means extending from 34 to 59 days, whereas the F_3 range of flowering time was from 45 to 71 days. Since both upper and lower limits of the range are displaced by like amounts and in the same direction, the general character of the distribution is not affected thereby.

TABLE IV

CLASSIFICATION OF MEANS OF F_4 "CONSTANT" FAMILIES FROM HOSHINO'S TABLES G–U

Classes	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59
White..	1	13	5	2		3	18	12	15	14	13	12	3													
Red or mixed		2	1	1		1	2	1	4	13	17	6	6	2		5	3	3	2	11	16	3	10	8	1	1
Totals .	1	15	6	3		4	20	13	19	27	30	18	9	2		5	3	3	2	11	16	3	10	8	1	1

Table IV shows unmistakably that the F_4 constant families fall into three natural groups, not four as Hoshino's hypothesis would lead one to expect. The

modes of these three groups fall at 35, 44 and 54, respectively, with frequencies of 15, 30 and 16, respectively. Each group is separated from the next adjacent by a gap (a class of 0 frequency). The modes of the early and late groups correspond closely with those of the uncrossed early and late parent varieties, which in this season had modes at 35 and 56, respectively. The third and largest group has its mode almost exactly midway between the modes of the early and late groups, with 8 intervening classes below it and 9 above. It would be difficult to imagine a finer example of a stable intermediate class produced by hybridization. For it will be remembered that every part of this population is stable, since it includes only families shown by Hoshino's breeding tests to be reasonably constant, those which he actually pronounced "constant." From it, therefore, one would need only to choose families of the desired flowering time, in order to have a complete succession of varieties from very early to very late.

But it may be asked, is the middle group possibly an "early intermediate" group of Hoshino's formula aaBB separated from the later groups by a class of minimal (0) frequency, as in the F_2 distribution? If so it should contain very few red-flowering families, no more indeed than the early group itself, since each would obtain red-flowered families only by cross-overs. Inspection of Table IV shows that this hypothesis is untenable. The truly hybrid origin of the middle group is shown by the large number of red-flowered or mixed families which it contains. Nine out of 12 of the F_3 families which contributed to the production of the middle group contained red-flowered or mixed red and white families. The middle F_4 group itself contained 90 white-flowered to 52 red-flowered or mixed families, whereas the early group contained 21 white: 4 red, and the late group contained only red-flowered or mixed families.

Hoshino observed that the flowering time of F_1 plants was close to that of the late parent, being only 2 or 3 days

earlier, and considers this to be evidence of dominance, but I am inclined to think it should be interpreted differently, for F_2 plants having the genetic properties of F_1 plants are in some cases at least (Hoshino's Tables T and V) much earlier in flowering time, being in fact almost exactly intermediate between the parent races, although in one family (S) the F_2 plant was late like F_1, but its F_3 and F_4 descendants covered the entire range, as did those of T and U. I am inclined to interpret the inheritance as truly *intermediate* and to explain the lateness of F_1 and of an occasional F_2 individual as due to physiological non-genetic causes. Recent observations made on size inheritance in guinea-pigs together with certain observations recorded by Hoshino lead me to this conclusion. When the small *Cavia cutleri* is crossed with the relatively large guinea-pig, the F_1 hybrids are larger than either parent, but the F_2 hybrids as a group are close to intermediate and only a little more variable than F_1. A stimulus due to crossing makes F_1 larger than its genetic constitution would otherwise make it, but the added size due to this stimulus does not persist to any great extent beyond F_1. Hoshino's F_1 peas probably possess a similar vigor due to crossing, which quickly disappears in later inbred generations. If this vigor due to hybridizations causes extra growth it may delay flowering time, for Hoshino, confirming Keeble and Pellew, has shown that late-flowering plants have longer internodes than early-flowering ones.

Hoshino, as we have noted, divides his F_2 families into two portions, early-flowering (chiefly white) and late-flowering (chiefly red). But we have seen reason to think that the F_4 families fall naturally into three portions, early, medium and late, and it is possible to divide the F_2 families in a similar way, though of course somewhat arbitrarily, classing as early those falling within the range of the early parent or a little beyond it, and as late those which fall within the range of the late parent, while those which lie between are placed in

the middle class. When the F_2 families are treated in this way we find the distribution of white-flowering and red-flowering plants shown in Table 5.

TABLE V

DISTRIBUTION OF WHITE- AND OF RED-FLOWERING AMONG THE F_2 PLANTS

Hoshino's Table	Early		Medium		Late		Total
	White	Red	White	Red	White	Red	
Table 2	49	13	20	114	7	168	371
Table 8, A_1	10	3	10	62	1	11	97
Table 8, A_2	17	2	2	20	3	25	69
Table 8, B_1	4	0	6	25	0	7	42
Table 8, B_2	17	0	7	37	5	30	96
Table 8, C	26	24	3	37	5	45	140
Total	123	42	48	295	21	286	815
Per cent. white	74		14		6.8		

Hoshino uses his F_2 tables as a basis for calculating the strength of the coupling between earliness and flower color, and concludes that the coupling is approximately 7:1. If, however, F_2 is divided as in Table 5, the coupling appears to be less strong, probably about as 4:1 or 5:1. The percentages of white-flowered plants expected in each group on various integral coupling ratios are as follows:

Coupling	Early Group	Medium Group	Late Group
4:1	64%	16 %	4 %
5:1	69%	14 %	2.8%
6:1	73%	12 %	2 %
7:1	76%	10.9%	1.5%
Observed	74%	14 %	6.8%

It will be observed that the percentage of white-flowered plants in the early group indicates about a 6:1 coupling ratio, but in the medium group, it indicates a 5:1 ratio, while in the late group it would indicate a 3:1 ratio. Much uncertainty exists as to the classification of many of the F_2 plants as regards flowering time, because of irregular and delayed germination. Undoubtedly the classes early, medium and late overlap, so that not much

reliance can be placed on the categories adopted. But the figures given indicate that Hoshino has estimated too high the coupling strength, and that more probably it does not exceed 5:1. This is not due to any inaccuracy in Hoshino's calculations, but would follow only if the hypothesis suggested in this paper is substituted for Hoshino's hypothesis.

If I have correctly interpreted Hoshino's observations, flowering time in peas is clearly a Mendelian unit-character, entirely devoid of dominance, so that a strictly intermediate hybrid form is the commonest end-product of a single cross between early and late varieties. Further, segregation is imperfect so that blending results, which becomes more and more complete with each generation of inbreeding. From the incompleteness of the blending in the F_1 zygote and so the imperfection of the segregation in the F_1 gametes, it follows that many different types of F_2 zygotes are produced, some of which are practically constant (homozygous) particularly those at either extreme of the series (the "early constant," "pseudo-early constant," "late constant" and "pseudo-late constant") and also at two intermediate points ("intermediate early" and "intermediate late").

Other F_2 zygotes, resulting from the union of gametes quite dissimilar, produce a highly variable F_3 progeny, but one which will give rise to F_4 families individually less variable for two reasons: (1) because the process of blending continues and so gametes produced by the same zygote become more like each other than were the parent gametes of that zygote, and (2) because heterozygotes under self-fertilization tend to produce about 50 per cent. of homozygous offspring, while homozygotes produce only homozygous offspring.

The entire population therefore will in accordance with recognized Mendelian principles gradually resolve itself into relatively constant self-fertilizing lines. But because of the slow but continuous blending which occurs, these pure lines will in a very few generations form a complete

gradation of forms connecting one parental mode with the other. The most numerous of these intermediate forms will in F_4 and later generations be that which is midway between the modes of the respective parents.

This case throws a flood of light on the nature of blending and of Mendelian inheritance and of their relations to each other. In typical Mendelian inheritance determiners of allelomorphic characters may meet each other generation after generation in a common zygote, separating again in gametogenesis without apparent modification of either in consequence of their conjugation in a heterozygote. This is well illustrated in the color inheritance of animals and plants.

In typical blending inheritance the determiners of contrasted parental conditions apparently blend into a determiner of intermediate character, the gametes formed by an F_1 individual being practically as uniform in character as those of either parent individual. Blending is illustrated in the inheritance of ordinary size differences in birds and mammals (Castle, 1916).

A third type of inheritance must now be recognized which is a compromise between these two, for it exhibits Mendelian segregation of the contrasted parental conditions but with modification due to partial blending of the unlike determiners in the F_1 zygote. The blending increases and evidences of segregation decrease with every generation during which the contrasted characters remain in conjugation. Consequently with every generation of inbreeding or self-fertilization following a cross of this sort, a stable intermediate class is more and more closely approached until its realization is complete. See Marshall (1916) on the Corriedale breed of sheep. The existence of this third type of inheritance was pointed out by Castle and Forbes (1906) in the case of hair-length in guinea-pigs and by Castle (1906) in the case of polydactylism in guinea-pigs. The opinion was then expressed that "more characters fall in this category than in any other." Hoshino's observations on flowering time in peas (if I

rightly interpret them) fully establish the existence of this third type of inheritance. Incidentally they indicate that the Nilsson-Ehle principle of multiple determiners to explain blending inheritance is quite superfluous.

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THE OCCURRENCE OF THREE RECOGNIZED COLOR MUTATIONS IN MICE

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THE common wild mouse, *Mus musculus*, has a type of coat-color pattern well known to geneticists as being characteristic of almost all wild species of rodents. This pattern is commonly called the "agouti" pattern. It consists in a "ticking" or "banding" of the hairs of both dorsal and ventral surfaces. On the dorsal surface each hair has a subapical band containing yellow pigment. The tip of the hair is black pigmented, while the half proximal to the yellow band is dark, containing both black and brown pigment granules. The general effect of the dorsal surface, when considered as a whole, is dull, brownish gray. The ventral surface is distinctly duller and paler than the dorsal. The distal half of the hair is lightly pigmented with black and occasionally with some yellow pigment, while the proximal half is much more heavily pigmented with black and brown granules. The general impression, conveyed by the ventral surface, is dull faded gray. This fact has led to the adoption by various investigators of the term "gray-bellied agouti" to describe the wild mouse color pattern.

In addition to this gray-bellied agouti another type of agouti house mouse has been reported as occurring wild in certain localities. This type has been used in genetic investigations by Cuénot and by Morgan.[1]

The chief difference between the gray-bellied agouti and the aberrant type is that the latter has, generally speaking, more yellow and brown and less black pigment. This is especially true of a band of hairs which runs laterally along

[1] Morgan, noticing that Cuénot's mice of this variety had a small patch of reddish brown hair between the front legs while his own mice had not, has suggested the possibility that the variety with which he worked was not identical with that described by Cuénot. I have at present animals of this color variety with and without the spot and therefore hope soon to have a definite answer to this question.

the boundary of the lighter ventral hairs between the front and hind legs. This streak is very reddish in color and adds to the lighter and warmer coloring of the dorsal surface. Ventrally, the tips of the hairs are to all extents and purposes unpigmented and this produces a white or nearly white ventral surface. The proximal half of the hair is dark pigmented, a distinction between this type of white area and that found in spotted animals where the hair is white throughout its length. This type of agouti has been given the name of white-bellied agouti (gris à ventre blanc; Cuénot).

In crosses with gray-bellied agouti the white-bellied form is epistatic. It can be obtained in a homozygous condition and is, as stated by Cuénot and by Morgan, one of a series of four allelomorphic forms of coat pigmentation.

It is the appearance of this epistatic "white-bellied" agouti in two experiments involving gray-bellied agouti and non-agouti mice that I wish to record.

EXPERIMENT *A*

In 1913, a cross was made between wild gray-bellied agouti mice and a race of dilute brown mice which I had started in the laboratory of the Bussey Institution.

The agouti race used was directly descended from wild animals without any out crossing with any domesticated mice. It therefore represented a stock of wild mice raised for several generations in captivity.

From this stock of agoutis I have recorded 531 young. All of them were gray-bellied agoutis[2] of the type that one might catch wild in any house. A certain amount of variation in intensity of pigmentation occurred among these mice. This variation, however, was not different so far as could be observed from that shown by a considerable number of specimens of this species from any single locality, judging from skins in the museum of comparative zoology of Harvard University. Among these skins, and

[2] The brown agoutis recorded later in this paper are of the dark or "gray-bellied" type.

among the mice raised from the gray-bellied agouti stock, there was not even an approach to the white-bellied agouti type.

The dilute brown animals which were used belonged to a race descended from a single pair of dilute brown mice which had been tested by suitable matings in order to establish their homozygosity. These animals in common with other "non-agouti" varieties of mice lack all visible traces of the agouti pattern and are with the exception of a slightly lighter ventral than dorsal surface uniformly pigmented throughout.

The cross between these two races, then, was one between a gray-bellied agouti and a "non-agouti" race. Both races used as parents were therefore color varieties which were hypostatic to the white-bellied agouti type and which for this reason could not carry that pattern as a recessive.

Four females of the dilute brown race were crossed with a gray-bellied agouti male 131 and produced 26 gray-bellied agouti young as shown in Table I.

TABLE I

GRAY-BELLIED AGOUTI

Mating	
♀ b1 × ♂ 131	6
♀ b2 × ♂ 131	8
♀ b3 × ♂ 131	6
♀ b4 × ♂ 131	6
	26

The F_1 gray-bellied agoutis were more intensely pigmented than their gray-bellied agouti parents. This deepening of color has frequently been observed in crossing wild agouti varieties with tame races and is probably due to modifying factors introduced by the particular tame race used or else to a general acceleration of pigment production due to heterozygosis.

The F_1 animals were crossed *inter se* and 285 F_2 young were raised. Of these 14 died at too early an age to have their color recorded.

In an earlier paper (1913), I have reported a cross similar to the one here recorded and have shown that three pairs of alternative mendelizing factors are involved. These are:

A—gray-bellied agouti.	a—non-agouti.
B—black pigment.	b—no black pigment.
D—intense pigmentation.	d—dilute pigmentation.

The gray-bellied agouti parent was homozygous for the factors for gray-bellied agouti, black and intensity being AABBDD in zygotic formula. The dilute brown parent was homozygous for the hypostatic conditions, non-agouti, no black pigment, and dilute pigmentation, and was, therefore, aabbdd in formula.

The F_1 gray-bellied agoutis would of course be heterozygous for all three pairs of factors and would be AaBbDd in formula. When such F_1 hybrids are crossed together, eight color classes of young are expected in a 27, 9, 9, 9, 3, 3, 3, 1 ratio. As will be seen from Table II, this result was approximated with the addition of a ninth and unexpected color variety, the white-bellied agouti.

TABLE II

	F_2 Generation									
Mating	Black Agouti Gray-belly	Black Agouti White-belly	Black	Brown Agouti	Dilute Black Agouti	Brown	Dilute Black	Dilute Brown Agouti	Dilute Brown	?
A 32×13	4	—	—	2	1	2	—	1	—	1
B 14×17	2	—	—	—	1	—	1	2	—	2
C 8×20	8	—	6	3	3	1	1	—	1	6
D 16×18	4	—	—	4	1	—	1	—	—	—
E 33×22	2	—	—	—	—	—	—	—	—	5
F 21×20	15	1	2	4	3	3	3	2	1	—
G 9×31	10	—	4	8	4	—	3	2	1	—
H 7×31	3	—	4	1	2	—	1	—	—	—
I 6×18	16	—	3	3	3	2	3	4	—	—
J 23×17	9	—	3	3	2	—	—	—	—	—
K 30×19	24	—	6	4	5	1	2	2	2	—
L 15×13	15	—	3	3	4	1	—	—	—	—
M 12×22	12	1	4	2	3	1	2	1	—	—
Obtained numbers	124	2	35	37	32	11	17	14	5	14
Expected on three factor basis	116.1	—	38.7	38.7	38.7	12.9	12.9	12.9	4.3	—

It will be noticed that the approximation to the expectancy on the basis of three pairs of Mendelian factors is extremely close. The occurrence of the two "white-bellied" agouti young is the matter of especial interest. These two young, one a male the other a female, are apparently identical with the gris à ventre blanc of Cuénot, judging from his description.

The question may quite naturally arise as to the possibility that the white-bellied agouti animals in F_2 had arisen by an accidental cross with some white-bellied agouti male in an adjoining cage. This possibility, however, is obviated by the fact that the F_2 generation white-bellied agoutis appeared in different cages at the Harvard Medical School where there had been *no other white-bellied agoutis* for more than a year before the appearance of the mutant animals. The two F_2 white-bellied agoutis must then be considered as true mutants epistatic to the type from which they originated.

Further evidence is provided by the F_3 generation, a tabulation of which is given below.

It will be observed that there is one "white-bellied" agouti among the 624 animals comprising the F_3 generation. This one mutant occurred as a descendant of a family of F_2 animals shown in Table II, mating J. This mating in F_2 gave 9 black agouti and 3 brown agouti young, none of which were "white-bellied" and the mutation therefore originated in a gamete formed by one of the F_2 generation animals while the other two white-bellied agoutis originated as mutations in the gametes of F_1 animals.

This recurrence of the mutation is a matter of considerable interest and indicates a germinal change, the occurrence of which is largely irregular, having some underlying cause or causes which become operative in animals of this particular hybrid race.

That hybridization between the same wild agouti and dilute brown races does not always produce this or any other recognizable mutation from the eight expected color

TABLE III

		F3 Generation										
F2 Parents	F3 Mating	Black Agouti Gray-belly	Black Agouti White-belly	Black	Brown Agouti	Dilute Black Agouti	Brown	Dilute Black	Dilute Brown Agouti	Dilute Brown	Pink-eyed Black Agouti	Pink-eyed Dilute Black
M	164×161.....	2	—	—	2	—	—	—	—	—	—	—
	163×161.....	3	—	3	—	—	1	—	—	—	—	—
	72×76.....	—	—	—	—	20	—	14	4	—	—	—
	163–164×161.....	14	—	1	11	—	2	—	—	—	—	—
L	331×335.....	—	—	—	2	—	—	—	—	—	—	—
	167×168.....	10	—	10	5	2	2	—	1	—	—	—
	165×168.....	2	—	1	—	—	—	1	—	—	—	—
	89×86.....	12	—	—	—	6	—	—	—	—	—	—
	84×86.....	16	—	—	—	9	—	—	—	—	—	—
	84–89×86.....	30	—	—	—	3	—	—	—	—	—	—
	165–167×168.....	7	—	5	2	2	1	—	1	—	—	—
K	280×320.....	1	—	1	—	—	—	—	—	—	—	—
	130×135.....	3	—	2	2	—	2	—	—	—	—	—
	57×56.....	3	—	2	7	1	—	—	1	2	—	—
	55×56.....	2	—	6	—	—	—	1	—	—	—	—
	51×56.....	4	—	6	2	—	2	—	—	—	—	—
	51–57×56.....	3	—	4	1	2	1	1	2	1	—	—
J	145×142.....	4	—	—	2	—	1	1	3	1	—	—
	144×142.....	3	1	1	—	—	—	—	1	—	—	—
	91×93.....	10	—	17	—	2	1	3	—	—	—	—
	90×93.....	—	—	35	—	—	8	6	—	5	—	—
	144–145×142.....	3	—	1	—	3	—	—	1	—	—	—
F	136×137.....	9	—	1	3	—	—	—	—	—	—	—
	136–140×137.....	6	—	1	2	—	1	—	—	—	—	—
G	185×182.....	—	—	—	42	—	10	—	2	—	—	—
H	110×106.....	3	—	5	—	3	3	5	2	—	—	—
	102–103×101–105.	3	—	1	—	—	—	4	—	—	3	2
I	172–174×171.....	16	—	—	10	17	—	—	14	—	—	—
	80×78.....	4	—	1	—	—	—	—	—	—	—	—
	79×78.....	6	—	7	—	—	—	—	—	—	—	—
	79–80–82×78.....	16	—	20	—	—	—	4	—	—	—	—
	79–80×78.....	5	—	6	—	2	—	—	—	—	—	—
		200	1	137	93	72	35	40	32	9	3	2

varieties shown in Table II, is proved by the following experiments.

In 1911 a parallel cross was made between wild agouti and dilute brown races. The making of this cross I have recorded (1913; p. 55, Cross 20*a*; p. 52, Cross 10*a*, etc.) in a previous paper. The first hybrid generation consisted of gray-bellied black agouti animals, 8 in number. The second hybrid generation consisted of 55 animals which were of the expected color varieties. In the third hybrid and ensuing generations, including back crosses, I have recorded more than 4,500 young. None of these animals

were white-bellied agoutis or indeed of any but the expected eight color varieties. This proves conclusively that the hybridization of these two races does not necessarily cause the white-bellied mutation to appear.

The fact that the white-bellied mutation has occurred in wild races of *Mus musculus* as shown by the capture of white-bellied mice would indicate that this mutation does not *necessarily* depend for its origin upon the presence in the germ cell of any of the recognized color factors *in a heterozygous condition.*

The test by breeding of the white-bellied agoutis obtained in the F_2 and F_3 generations has been attempted. One of F_2 generation mutants has not yet been bred, but the other F_2 and F_3 white-bellied agoutis have shown that their color pattern was epistatic to gray-bellied agouti and non-agouti. In these respects, the mutants have behaved in a manner identical with the gris à ventre blanc mice of Cuénot or the white-bellied agoutis of Morgan.

The observed facts therefore prove clearly the origin of this epistatic white-bellied agouti mutation at three different times in the course of this experiment.

It is interesting to note that there has been no selection in the direction in which the mutation occurred as was the case in the experiments of Castle and Phillips (1914) on hooded rats.

Although the white-bellied agouti may easily be considered merely an increased state of activity of the agouti factor beyond the gray-bellied stage, it is certain that it arises, not as the product of a series of small gradual changes, but suddenly and distinctly, without warning, and that after its appearance it behaves at once as a mendelizing character.

Taking into consideration the facts now recorded concerning this particular mutation we can say that its origin is apparently not solely dependent upon any of the known genetic processes. Inbreeding, hybridization, selection, none of them is indispensable to the occurrence of the mutation. The underlying cause or causes are at present clearly outside the bounds of analysis.

EXPERIMENT *B*

In 1912 I started, on a small scale, an experiment which had for its object the modification of the agouti pattern by repeated crossing with black (non-agouti) animals. It was thought that if the gametes of agouti animals showed quantitative variation in the factor underlying the agouti pattern repeated crossing with non-agoutis would succeed in decreasing the amount of the agouti character. It follows that if this was accomplished by contamination between the agouti gamete and the non-agouti (black) gamete, the non-agouti animals should show increasing traces of the agouti character as the agouti animals showed a diminution of that character.

The method followed was to cross black agouti animals derived from a sooty yellow stock with blacks from the same stock and selecting the blackest agoutis from each litter repeat the process. All the agoutis used were thus heterozygous for the agouti factor. This process was repeated for more than seven generations during which approximately 400 young were recorded. From the outset two facts were evident. *First, the agoutis grew distinctly blacker: second, there was no corresponding sign of contamination on the part of the non-agouti animals, but they too grew blacker.* It appears, therefore, that there was no sign of modification of the *factor* underlying the agouti pattern; but that a race was isolated by selection which showed a distinct increase in depth of pigmentation. In this connection it is interesting to note that while the agouti animals grew blacker and the yellow areas in the hair decreased *in extent,* the yellow pigment itself was a deeper richer yellow than that of the ordinary black agoutis. The yellow pigment had been increased in *depth* while the black pigment was being increased in *depth* and in *extent,* two entirely distinct processes.

All the agoutis up to the third generation were gray-bellied agoutis usually having more dark pigment on the ventral surface than is found in wild mice. In the third generation there occurred an agouti with a distinctly yel-

lowish tinge to the under surface. The dorsal appearance of this animal was apparently the same as the other agoutis of this generation, a deep rich agouti with much black pigment. The ventral surface was, however, distinctly lighter in color than the other agoutis and showed a *decrease in dark pigment* as compared to them.

The yellow-bellied variation reappeared in the immediate progeny of the original yellow-bellied female and a race of these animals was established. When single mating tests were made to determine its behavior it *was found that when yellow-bellied agoutis one of whose parents was a non-agouti were crossed with non-agouti, only yellow-bellied agouti and non-agouti young resulted.* This fact indicated that yellow-bellied agouti fell somewhere in the series of multiple allelomorphs recorded by Cuénot and by Morgan.

At about this time English "black and tan" mice[3] crossed with white-bellied agoutis were found to give among their progeny agouti young almost identical with the yellow-bellied agoutis described above.

It at once suggested itself that the yellow-bellied agoutis occurring in my selection experiment were really *white-bellied agoutis* with one or more modifying factors which encouraged a higher degree of pigmentation than is normally found. This increase in an oxidation process would account for yellow pigment appearing in the tips of the ventral hairs which in ordinary white-bellied agoutis are unpigmented.

This supposition was further favored by the fact that as certain of the yellow-bellied agoutis grew to be old mice they showed a diminishing depth of pigmentation, *and developed typical white-bellied agouti coat color.* In old age they were not visibly different from some of the darkest white-bellied agoutis descended from a male of this variety kindly sent me by Professor Morgan.

[3] Black and tan mice are a very dark type of yellow. Only a small amount of yellow appears on the sides, while the ventral surface is a deep rich reddish yellow. Black animals descended from black and tans are coal black with deep black ears, feet and tail.

One breeding experiment is interesting in showing the probable identical nature of the factor underlying the yellow-bellied and white-bellied agouti types. Two yellow-bellied agouti females known to carry non-agouti were mated with a white-bellied agouti male known also to carry non-agouti. On the supposition that the white-bellied and yellow-bellied mutations are the same, this mating should produce two types of young, agouti (either yellow or white-bellied, not both) and non-agouti in a 3:1 ratio. If on the other hand yellow-bellied agouti depended on a distinct factor falling in the multiple allelomorph series hypostatic to white-bellied agouti, the mating should produce three types of young, white-bellied agouti, yellow-bellied agouti, and non-agouti in a 2:1:1 ratio. Finally, if *yellow-bellied* agouti depended upon an *independent modifying factor which was acting upon a gray-bellied agouti animal,* one of two results should be obtained. If the factor was epistatic we should have white-bellied agoutis (plus the modifier), yellow-bellied agoutis and non-agoutis in a 2:1:1 ratio. If the modifier was hypostatic in the cross, white-bellied agouti, gray-bellied agouti and non-agouti young should occur in a 2:1:1 ratio. The three litters obtained have totaled 19 young; 11 white-bellied agouti and 8 non-agouti. The two classes of young seem to indicate strongly the correctness of the view that yellow-bellied agoutis are in reality white-bellied agoutis plus a darkening modifying factor or group of darkening modifiers the nature of which is not yet sufficiently clear to allow further description.

The point of extreme interest is that the white-bellied agouti mutation has occurred in a race which was being selected in directly the opposite direction from that taken by the variation. White-bellied agoutis represent a *stronger* agouti factor than the gray-bellied agouti factor, while every effort was being made in this experiment to weaken the gray-bellied agouti factor. It appears that the occurrence of the white-bellied mutation in experiment *A,* above recorded where *no selection* was being

exercised, and the independent occurrence of the same mutation in experiment *B against* the course of selection are evidences that *the direction of mutation is largely if not entirely independent of selection and that the occurrence of the plus mutant rat in the plus selection series of Castle and Phillips before referred to, is in all probability a matter of coincidence rather than the result of selection as they have hinted.*

Pink-eyed Mutation

It will be noticed in the table showing the F_3 generation of the previous experiment (*A*) that among the 624 young recorded, 5 are pink-eyed. The pink-eyed mutation is hypostatic to dark-eye color and has been known for some time (see especially Castle and Little, 1909, Durham, 1911). These young all appeared in a single pen in which were two females and two males all of a single litter. Eight dark-eyed and five pink-eyed young were produced by these mice.

Up to the appearance of the pink-eyed young it was not suspected that the pink-eye factor in any way entered into the experiment. It was certain that one parent stock consisted of pure wild mice and that the pink-eye factor, which is a recessive, was not brought into the cross by this parent.

The pink-eye mutation, being recessive, could easily have been latent in the cross for some time without the combination of gametes necessary for its manifestation in a zygote having been realized. The dilute brown animals from which the second parent race was descended had originally been tested by breeding and had been found to be entirely free from the recessive pink-eye factor. Since, however, this mutation *appeared in only one F_3 family* it seemed distinctly unlikely that the original wild male, 131, which had been used as the male parent of all families, was the animal through whose gametes the mutation came into the cross. On the other hand, as there were four dilute brown females (Nos. b1, b2, b3, b4,) used in

producing the F_1 generation, it seemed quite likely that one of these first introduced the pink-eye factor.

If such was the case the animal in question would be female No. b2. This mouse was the dilute brown ancestor of the particular group of F_2 animals which in turn were the parents of the pink-eyed mutant individuals.

The suggestion that the dilute brown parent is the animal which introduced the pink-eye factor is supported by the fact that *at about the same time at which the mutation appeared in the F_3 generation of this experiment it also appeared within the "pure" dilute brown race.* This makes it extremely probable that the mutation had already occurred within the dilute brown race and was brought into the cross by a single dilute brown female which because of the fact that it was dark-eyed concealed the presence of the recessive pink-eyed factor which in all probability existed in approximately half its gametes.

Unfortunately at the time that this mutation appeared in F_3 the dilute brown great grandparent had died. A breeding test was therefore impossible in order to ascertain whether she actually carried the pink-eye factor in one half of her gametes.

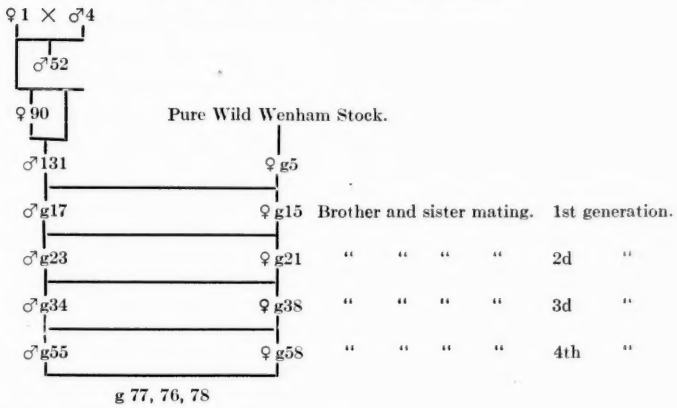
EXPERIMENT *C*

Another mutation, this time of the black producing factor, has occurred in a stock of pure wild mice, the original individuals of which were caught either in Wenham, Mass., or in Forest Hills, Mass., in 1912.

The particular family in which this mutation occurred is shown in Table IV. As will be seen, two wild mice both caught at Forest Hills, Mass., were bred together, these are female 1 and male 4. They and all their descendants, unless especial mention of the fact is made, will be considered black agouti in color. That is to say they were in appearance ordinary wild house mice. Female 1 and male 4 gave among their progeny male 52, who was crossed back to his mother and thus gave rise to female 90. She in turn was crossed back to her father, male 52, and from this mating male 131 was obtained.

TABLE IV

ANCESTRY OF BROWN-AGOUTI MUTANTS



8 Black agouti.
3 Brown Agouti.

Male 131 was used in many crosses, one of which was with female g5. This female was a pure wild mouse taken from a stock pen of wild mice which for several generations had been reared in captivity by Dr. J. C. Phillips at Wenham, Mass. From this mating came 6 black agouti young in a single litter. Two of these young, female g15 and male g17, brother and sister, were mated together and produced 11 black agouti young, two of which, male g23 and female g21, were mated together. This brother and sister mating gave 16 black agouti young from which another brother and sister mating, male g34 and female g38, was made. From this pair was obtained 13 black agouti young, among which were male g55 and female g58, the parents of the mutants. These two mice male g55 and female g58 have had three litters of young. The first born in December, 1915, consisted of three animals, male g77 *Brown Agouti*, female g78 *Brown Agouti* and female g76 *Black Agouti*. The appearance of the brown agoutis was entirely unexpected and was thought to be possibly due to an error in records. A sec-

ond litter however, born in February 1916 after the parents had been under careful observation, consisted of two black agoutis, a male and a female, and *one brown agouti a female*. The third litter of five is all black agouti.

This is a clear case of mutation within a closely inbred race, and is interesting to contrast with Experiment *A* already referred to, in which a mutation occurred in hybrids.

There is one fact of possible interest in connection with the mutations recorded in Experiment *A* and in this experiment. Male 131, black agouti, is a common ancestor of all the races in which the mutations occurred. It has been shown that the evidence is *against* his having introduced the pink-eyed mutation and that this probably came from the dilute brown race.

For the other two mutations, however, the white-bellied agouti and the brown agouti types, it is theoretically possible that male 131 possessed or transmitted an instability of germplasm which has manifested itself in the cropping out of these mutations among his descendants. Fortunately the stock within which the brown agouti mutation arose is being carried on in single pair, brother and sister, matings. By this method we should be able to recognize mutations at the earliest possible moment after their occurrence.

SUMMARY

To sum up the facts above recorded it may be stated that:

1. A previously recorded mutation of the gray-bellied agouti pattern, known as white-bellied agouti, has arisen in two experiments on color inheritance in mice.

2. In experiment *A* it has arisen independently three times in a hybrid race of mice.

3. In this experiment there has been no selection in the direction of the mutation.

4. In experiment *B* it has arisen once in an inbred race in which selection was being carried on.

5. In this race the mutation represents a variation in

exactly the opposite direction from that in which the selection was being made.

6. A recessive pink-eyed mutation has occurred in a closely inbred dilute brown race. A similar mutation has appeared in a hybrid race into which one animal from the dilute brown race probably introduced the mutation.

7. A mutation involving loss or suppression of the black producing factor has arisen in a stock of inbred wild mice. This has caused the appearance of brown agouti young.

8. The wild race in which this occurred is related to the hybrid race (see conclusion 2) in which the white-bellied agouti mutation appeared three times. The suggestion is offered that a tendency to germinal instability may have been transmitted by male 131 a common ancestor of both races.

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THE MECHANISM OF CROSSING-OVER III

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V. AN EXPERIMENT TO DETERMINE THE LINKAGE OF MANY FACTORS SIMULTANEOUSLY

A MORE exact knowledge of the interference of one crossing-over with another required an experiment, or series of experiments, in which the distance between the two points of crossing-over in cases of double crossing-over could be more accurately determined. In an experiment involving only three factors—A, D and H—if a double cross-over occurs, all that can be known is that crossing-over has occurred at the same time somewhere between A and D, and somewhere between D and H, but nothing can be known of the precise location and distance apart of the two points of crossing-over, except that they could not be further apart than A and H. On the other hand, if the inheritance of four points could be followed—say, A, D, F and H—then the distance between the two points of crossing-over could be determined a little more exactly, for a double crossing-over involving breaks between A and D, and between D and F, would cut out a shorter segment of the chromosome than one occurring in regions A–D and F–H. And the more numerous were the factors that could be followed—other things being equal—the more exact the determination would become. At the same time, it might be possible by comparing the results of a *series* of different experiments to arrive at the desired end with the three-factor method also. For example, the difference in frequency between the double cross-overs obtained in an experiment involving A, B, C and in an experiment involving A, B, D, must obviously be due to the double cross-overs involving regions A–B and C–D,[1] except in so far as these differences are due to the random deviation of different samples from each

[1] For region BD is made up of BC + CD. Therefore double crossovers involving AB and BD really consist of double cross-overs involving AB and BC plus those involving AB and CD. Consequently, if we subtract the number of double cross-overs involving AB and BC from the number involving AB and BD, we obtain the number involving AB and CD.

other, or to actual differences in the behavior of the chromosomes in the two experiments. As the last two influences seemed by no means negligible, and as the experiment involving many points at once gave a more direct and graphic picture of the results, it was decided to use this method of attack in preference. Moreover such an experiment incidentally afforded an opportunity of attacking certain other questions, such as the effect, on crossing-over, of having the two chromosomes different in regard to many factors. Meanwhile, the indirect method of attack would be followed by other workers, and the two sets of results could finally be used as checks upon each other.

The many factor method is in itself for several reasons very laborious, but this is compensated for by the fact that when the results are obtained they are the equivalent of an entire series of different experiments involving in turn the linkage of each factor with every other one, and indeed, the results are much more than the equivalent of these, for in the latter cases the linkages are obtained in different experiments, so that there is much more chance for error in determining the relation of one linkage to another.

It was evident from the outset, however, that there was one very important obstacle to be overcome in any study of linkage exact enough to give useful information regarding coincidence, and that the difficulty was especially great in the type of experiment contemplated. The difficulty referred to is "differential viability," for it is found that in nearly all experiments not involving the characteristics of seeds or other structures dependent upon the maternal organism for support, the individuals belonging to different genetic classes may be very differently equipped in respect to their ability to meet the struggle for existence. Thus, since the count generally takes note only of the individuals which survive, the ratios obtained may be very different from the ratios of the different classes of gametes. These discrepancies apply especially to forms like flies, the larval life of which can not be well controlled, and they are, of course, particularly great in crosses involving many factors at once.

Before considering the means by which differential viability may be reduced in crosses of multiple stocks, it may not be out of place to explain two methods I devised for getting a more correct estimate of the gametic ratio in back-crosses involving only two pairs of linked factors.

Let us say that the gametic ratio is r(AB):r(ab):s(Ab):s(aB). Assume that when A is present the viability of the flies is reduced so that only A′ per cent. of those which would otherwise survive, now come to maturity, and assume that factor B lessens the output to B′ per cent. of what it otherwise would be; similarly a and b, when present, lower the output to a′ per cent. and b′ per cent., respectively. Then the relative number of AB individuals which survive will be rA′B′ (per cent. marks are omitted for brevity); the relative number of Ab will be sA′b′, etc. The actual, observed, numbers will be some multiple (k) of these relative numbers; thus the number of AB individuals actually found will be krA′B′, the actual number of Ab will be ksA′b′, etc. It can now be shown that the true gametic ratio (r:s), which it was desired to find, may be derived by the formula

$$\sqrt{\frac{AB \times ab}{Ab \times Ab}}$$

(using Ab, ab, etc., to denote the number of AB observed, of ab observed, etc.), for, substituting the above values of AB, ab, etc., in this formula, we obtain

$$\sqrt{\frac{krA'B' \times kra'b'}{ksA'b' \times ksa'B'}} = \sqrt{\frac{k^2r^2A'B'a'b'}{k^2s^2A'b'a'B'}} = \sqrt{\frac{r^2}{s^2}} = \frac{r}{s}.$$

This formula should be used only when the smallest class has not a very large probable error, for, by multiplying the value of this class in the formula, we give the entire result a probable error proportional to that of the smallest class. Another objection to the formula is that it assumes that each factor produces the same specific lowering of viability, independently of whatever other factor it comes into combination with; this is not always true, since factors often produce different effects when in different combinations.

The two difficulties encountered above are largely avoided by the second method, which involves making two different kinds of crosses in preparation for the linkage determination: *i. e.*, cross AB by ab, and what may be termed the "contrary cross," Ab by aB. A back-cross of the F_1 from the first cross gives the gametic ratio r (AB) : r (ab) : s (Ab) : s (aB); and the other cross results in gametes showing the proportion s (AB) : s (ab) : r (Ab) : r (aB). Suppose that w per cent. of AB individuals are viable, x per cent. of ab, y per cent. of Ab, and z per cent. of aB. Then in the first cross the observed ratio would be r w (AB) : r x (ab) : s y (Ab) : s z (aB), and, in the second cross, s w (AB) : s x (ab) : r y (Ab) : r z (aB). The numbers actually observed in the crosses would be some multiple of these ratios, but a different multiple in the two cases. Thus we could designate the numbers actually observed in the first cross as krw(AB) : krx(ab) : ksy(Ab) : ksz(aB), and the numbers in the second cross as csw(AB) : csx(ab) : cry(Ab) : crz(aB).

In this case the ratio r : s may be obtained by the following formula:

$$\sqrt{\frac{AB_2 \times Ab_1}{AB_2 \times Ab_1}}$$

(using the symbol AB_1 to denote number of AB observed in the first cross, Ab_2 to denote number of Ab observed in the second cross, etc.). Now, the value of AB_1 has already been given as krw, of Ab_2 as cry, etc. Substituting these values in the above formula, we obtain

$$\sqrt{\frac{krw \times cry}{csw \times ksy}} = \sqrt{\frac{r^2kcwy}{s^2ckwy}} = \sqrt{\frac{r^2}{s^2}} = \frac{r}{s}.$$

Besides this formula involving AB and Ab, there are three similar formulas which will also give the gametic ratio, namely:

$$\sqrt{\frac{AB_1 \times aB_2}{AB_2 \times aB_1}}; \qquad \sqrt{\frac{ab_1 \times Ab_2}{ab_2 \times Ab_1}}; \qquad \sqrt{\frac{ab_1 \times aB_2}{ab_2 \times aB_1}}.$$

That formula should usually be chosen which contains the largest number of individuals in its smallest class, for this would usually have the least probable error.

This method makes no assumption as to an independent action of the different factors in reducing viability. It does assume, however, that for individuals with the same combination of factors there is the same degree of viability in the two experiments; this is not always true, since under different conditions of food, etc., individuals of the same genetic type may have very different degrees of viability; moreover, there are sometimes "invisible" factors present in one experiment but not in the other which influence viability and which are linked with the factors that are being studied. The assumption, nevertheless, is unavoidable. But it can be shown mathematically that any errors in the calculated values, due to assumptions made in following the formulas of either the first or the second method, are greatly reduced by using a combination of the two methods; namely, by making "contrary crosses," calculating the linkage value in each of them by means of the first method, and then taking the square root of the product of these two values.

In a cross involving three or more factors no formula corresponding to the one first given is possible, and before it is possible to use a formula corresponding to the second method, an increasingly large number of different kinds of crosses must be made, according to the number of factors involved. Still another method is, therefore, necessary in order to obtain fairly accurate results from crosses involving many factors, except in the rare case that these factors have very little differential effect on viability. The method devised is as follows:

The female, heterozygous for many factors, whose gametic output it is desired to study, is back-crossed, not to a multiple recessive male, but to one homozygous for all, or nearly all, the *dominant* factors (these are, in the case of flies, mostly the normal allelomorphs). All the offspring appear alike, then, in that they all show the dominant characters of their father (except in the case of sex-linked factors, which are transmitted by the father to his daughters only), and so all should be of the same viability, except for the insignificant effect of the recessive factors

present in heterozygous condition (and the effect of the one or two characters wherein the father may not have been dominant). Thus error due to differential viability may be held within safe bounds.

It may be objected, however, that we have, as it were, killed the patient in curing the disease—that there is no use in overcoming the discrepancies in the count due to differential viability, if we thereby eliminate the possibility of making any count at all, by making all the offspring appear alike! It is true that, in such an experiment, it is impossible to tell *by inspection* of any offspring, what maternal factors were present in the ova from which they sprang, since these factors are made invisible, so to speak, by the dominant factors brought in by the sperm. But the factorial composition of each of these offspring (which we will for convenience call "F_2") can be determined by breeding tests. The plan which was followed was to mate the F_2 flies, each in a separate bottle, to individuals containing the recessive factors. Thus whatever recessive factors were present in the eggs of the original heterozygous female ("F_1"), whose output it was desired to test, would become visible the generation after (in "F_3"). Whereas, in an ordinary linkage determination, each bottle produces a large number of flies, which need merely be classified according to their appearance, and counted—in this case, each of the offspring themselves requires to be mated and given a whole bottle to itself, and its progeny in turn ("F_3") must be examined. In other words, in ordinary cases, there is only one bottle necessary for a count of many flies, but in this case one bottle represents one fly of the count. The numerical relations existing between the flies ("F_3") hatching in one of these final testing-out bottles need not be determined, however; that is, these flies need not be counted; all that is necessary is a "qualitative" determination of what recessive characters appear among them, in order to judge of the composition of their parent (F_2), which is the fly recorded in the count. Thus far 1008 of these test bottles have been recorded.

In preparation for this experiment the main task was to

secure stock that contained many mutant, linked factors at the same time. But, as was explained in the account of experiments with the third chromosome, it is necessary, in dealing with linked factors, to make the crosses in a particular way to secure a "multiple stock." Thus, it may be pointed out again here, a stock containing factors A, B, C cannot be obtained ordinarily by crossing stock A to stock C, and then crossing the double stock A C (produced in F_2, F_3, or F_4 from the first cross) to stock B; because it would require double crossing-over for the hybrid fly, containing A and C in one chromosome, and B in the other, to produce a gamete with A, B and C all in the same chromosome (assuming the factors to be linked in this order). If the linkage is tight such double crossing-over will never occur. But by first obtaining stock A B and then crossing this to C, stock A B C may be secured; for in the hybrid fly that contains A B in one chromosome and C in the other, a crossing-over between B and C will result in a chromosome that contains A, B and C, the link between A and B not having been broken.

In other words, the factors can only be added together in a certain order, owing to their position in the linkage chain. Just as in adding links to a chain, one or more factors cannot be wedged in *between* factors in another collection (except by double crossing-over); but if they lie *beyond* this collection, they may be added on, either singly or in a group. The information that had already been gained by Sturtevant, Morgan and Bridges concerning the order in which various factors lay, was therefore of great service in determining how the crosses should be made, to get the factors together, and besides this several double stocks of a sort that could be used in the present experiment had already been synthesized by them. But the progress of the experiment was very considerably retarded by the fact that the position of a number of the factors which it was desired to use had not yet been determined. These comprised bifid and forked in chromosome I and dachs, jaunty, curved, arc and balloon in II. (The exact position of jaunty with respect to black, and of

balloon with respect to speck is still unknown.) Various "trial and error" matings were therefore made in the hope of getting these unplaced factors in suitable combinations, and crosses were also undertaken to secure such data in regard to their position as would be useful for the purpose in view. These attempts were often cut short, owing to the information which was meanwhile being accumulated by the other workers, but before the latter information was obtained the positions of bifid, forked and dachs had been determined, and several multiple stocks that were later used had been made up.

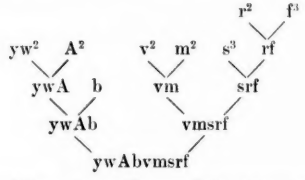
We may now consider specifically what combinations of factors were actually employed in the experiment and what special methods were used for securing and maintaining these combinations.

In the case of the first chromosome, it was desired, for the final linkage determination, that the heterozygous flies, whose gametic output was to be tested, should contain most of the recessive factors (which are usually the mutant ones) in one chromosome, and the dominant (usually normal) factors in the other, for it was considered worth while to test the possibility that a chromosome containing so many mutant factors might behave abnormally. Furthermore, if the commonly accepted belief were correct, that recessive factors are "absences," it was possible that the chromosome with many recessive factors might be shorter than the other, and that linkage disturbances might arise for this reason. Two stocks were, therefore, made up, of the factors in the first chromosome, to supply these two kinds of chromosomes for the heterozygous females to be tested. One stock contained the mutant factors for yellow body color, white eyes, abnormal abdomen, bifid wings, vermilion eyes, miniature wings, sable body color, rudimentary wings, and forked spines. All of these factors are recessive except abnormal abdomen, which is only partially and irregularly dominant. The other stock contained only the mutant factors cherry eye, club wing, and bar eye. Cherry is an allelomorph to the factor for white eyes carried by the other chromosome,

and is dominant to it, though not completely; club is recessive; bar is dominant (somewhat incompletely).

The reason that club was not put into the series with the other recessives is that it was discovered (by Bridges) after this series had already been put together, and so it would have required taking the stock apart again, or else obtaining a rare double cross-over, to wedge club into this series. It was a valuable factor to have in the experiment, however, since it lay in a region of the chromosome where there were no other mutant factors to give data as to crossing-over. Accordingly, it was inserted in the other series. It will be observed, however, that, in spite of this, one chromosome contains 7 more dominants than the other.

The order of the above factors is y, w or c, A, b, c_1, v, m, s, r, f, B_r. In making up the first stock the factors were put together as follows (omitting from consideration all trial or discarded combinations):



Of course the putting together of factors from two stocks, although shown above as only one step in each case, always requires several generations. Moreover, as will be seen below, these steps do not usually consist in getting the ordinary F_2 or back-cross, in the case of the complicated combinations. This is partly because of the serious obstacle which the poor viability of flies having many mutant characters presents to the making up of multiple stock, just as it does to the securing of counts from it; moreover, in the making up of stock, the sterility of such flies is an equally important difficulty.

These difficulties were overcome here in much the same way as they were in making the counts—namely, by keeping the stock, so far as possible, heterozygous. For ex-

[2] From Morgan.

[3] From Bridges.

ample, in the last step shown in the preceding diagram, where factors ywAb and vmsrf are to be put together, it was found that females of both of these kinds were extremely difficult to keep alive. It was, therefore, decided to mate a vmsrf male by a female which contained ywAb in one chromosome and normal factors in the other. Such a female would be easy to breed from, as the normal factors dominate. About half the daughters (let us call them F_1) would be of composition $\frac{\text{ywAb}}{\text{vmsrf}}$ (representing the mutant factors in the maternally derived chromosome on the upper line, those from the father on the lower line). All the daughters (F_1) would, however, appear normal, but if these F_1 females were bred in separate bottles, those of the desired composition $\frac{\text{ywAb}}{\text{vmsrf}}$ would be distinguishable from the others by their offspring (F_2). All bottles in which the parents (F_1) had not been of the desired composition could then be discarded. Next, among the offspring (F_2) of those females which proved to be of composition $\frac{\text{ywAb}}{\text{vmsrf}}$, it was necessary to select the ones which, by reason of crossing-over between b and v, contained all nine factors in the same chromosome (*i. e.*, ywAbvmsrf). But such individuals, if homozygous, never live long enough to mate, so great is the lowering of viability produced by all these mutant factors at once. Consequently, some method must be used of obtaining in this cross heterozygous individuals (F_2) which received this cross-over "nontuple" chromosome from their mother, and of distinguishing these from other individuals produced by the cross. The natural suggestion would then be that the F_1 females should be mated by normal males, and the F_2 which receive this cross-over chromosome could then be distinguished by breeding tests as their mother had been. The crossing-over desired, however, does not occur in more than one eighth of the flies, and so breeding tests designed to be certain of securing at least one individual of the required composition would have to be rather extensive. In this case, however, the desired F_2

flies can be "spotted" in another way, without breeding tests, and yet without making them homozygous for many mutant factors and thus inviable. The method used was to mate the F_1 females to bv males, which had been made up for this special purpose. The b v daughters (F_2) must be cross-overs, since in the F_1 mother b and v were in different chromosomes; moreover, a glance at the formula of the F_1 females will show that these cross-over chromosomes must have been formed of the left-hand end of the upper chromosome and the right-hand end of the lower. Thus these bv females contain a chromosome with all nine mutant factors (except in the case of the few double cross-overs). Since, however, they were homozygous in only two mutant factors, they could easily be bred.

A similar scheme was used in many of the other steps shown in the diagram representing combinations made in group I, and was also used frequently in group II. Owing to the fact that rudimentary winged females (group I) are practically sterile, devices of this sort had to be used in dealing with flies containing this factor from the very start, and the same may be said of flies with dachs legs (group II), since these also were found very hard to handle. In most of the other cases, however, it was not necessary to use such a method before several factors had been combined together, as flies homozygous for just two or three mutant factors were generally viable enough to handle. There would be no object in wearying the reader with a description of the exact way in which each of the steps was taken; it is the author's purpose only to explain the nature of methods used, giving only sufficient examples to make clear the details of any devices never previously employed that might be capable of application to other cases.

From the example of the cross involving bv, previously given, we may now generalize, and establish the rule that in making up, and also in keeping stocks containing many linked recessive factors, if the latter cause a marked lessening of fertility or viability, it is best to follow the practise of keeping the stocks heterozygous, by back-crossing them to stocks containing only the few recessive (or par-

tially recessive) factors necessary to show which offspring contain the desired cross-over or non-cross-over chromosome. In the example, this method was used in combining two stocks to make up a recombination stock. The same means is employed in maintaining the multiple stock after it has been synthesized. Thus, in the case of group I, the females containing in one chromosome the combination ywAbvmsrf (the "F_2" obtained above), were crossed to cc_1B_r males. In this way some daughters (F_3) are produced (which these were was determined by breeding tests) that received from their mother ywAbvmsrf, and from their father cc_1B_r. These F_3 females having the composition $\frac{ywAbvmsrf}{cc_1 \quad B_r}$, were then back-crossed to cc_1B_r males again, in order to maintain the stock. Since all the daughters (F_4) received cc_1B_r from their father, those which do not show these characters fully developed must have received from their mother factors near both ends of the chromosome containing the nine mutant factors. Therefore, except for the very few flies in which crossing-over occurred between w and y, which is at the very end, or in which double crossing-over occurred, all the light cherry, normal winged, partially bar eyed flies will have a composition like that of their mother, and may be bred in the same way, again to the cc_1B_r males, which now hatch from the same bottle. This then is a cross exactly like the preceding one, except for the few cross-over flies above mentioned. The latter may be detected, however, and their offspring discarded if the females are bred in separate bottles. This same cycle may be repeated generation after generation. Thus a continual supply is maintained of flies heterozygous for all these factors.

In making the linkage determinations, such flies are bred to normal or to bar males, and the female offspring, which are all alike in appearance except in respect to the partially dominant factors A and B_r, and which should, therefore, have had approximately equal chances for surviving, are individually tested for their contained characters. For the tests, the female need not be virgin, since, whatever kind of male is employed, the sons will show only

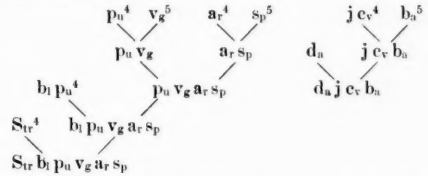
those sex-linked characters that their mother contained and they may therefore be used to determine the composition of their mother. As a matter of fact, however, males containing v were generally employed, so that v, if it had been present in the tested female, would appear in her daughters as well as her sons. This additional test for v was desirable because it is a factor which in a white, cherry, or bar eye it is difficult or impossible to detect.

Stock of the second chromosome was obtained, and is maintained, in an essentially similar way. Here the attempt was not made to put most of the mutant factors into one of the two chromosomes of the heterozygous females to be tested. This was partly because an experiment of this sort with one chromosome would seem sufficient. Moreover, it was harder to make up multiple stocks of the second chromosome, since the order of certain factors had not at first been well determined, and since, besides, it takes a greater number of generations to put non-sex-linked factors together into the same stock than it does to put sex-linked factors together. For, if two recessive stocks of chromosomes II are crossed, the F_1 males, in which crossing-over never occurs, transmit the recessive factors of only one stock to each son and daughter. The latter then can not be homozygous for both sets of recessive factors, and so it is impossible to pick out, except by further breeding, those that received both sets from the mother. But as in the case of the bv illustration given, if a male with C D is available to cross with the "F_1" hybrid female $\frac{ABC}{DEF}$, the "F_2" individuals showing both characters C and D must have the composition $\frac{ABCDEF}{CD}$, and so the desired cross-overs may be picked out immediately in F_2.

A somewhat similar scheme, often especially useful for obtaining desired combinations in a non-sex-linked group, involved making use of cross-overs that break combinations already obtained. This too may be shown by an example. It was desired to obtain a stock containing the second chromosome factors dachs legs, jaunty wings, curved wings, and balloon wings. Dachs black stock al-

ready had been made up, as had j c_v b_a. These two stocks were crossed together, and the F_1 was back-crossed to d_a b_1. In the F_2 generation all the dachs that are *not* black are cross-overs in the region between these two factors, and so must contain, in the same chromosome with dachs, instead of black, the factors j c_v and b_a, for black is in such a position in the chromosome that a cross-over between d_a and b_1 must nearly always be a cross-over between d_a and j c_v b_a.

In the case of the second chromosome the individuals tested for linkage contained, in one chromosome, the factors streaked thorax, black body color, purple eyes, vestigial wings, arc wings, and specked thorax, and in the other chromosome, the factors dachs, jaunty, curved and balloon. The order of these factors is as follows: S_{tr} d_a b_1 p_u v_g c_v a_r s_p b_a. The way in which they were combined is as follows:



As d_a j c_v b_a is very inviable it is kept in heterozygous condition by back-crossing, in each generation, normal appearing males of the composition $\frac{d_a\ j\ \ c_v\ \ b_a}{b_1\ p_u\ v_g\ a_r\ s_p}$ (called $\frac{4}{5}$ for short) to b_1 p_u v_g a_r s_p females (called 5). Since there is no crossing-over in the male, all the offspring are either apparently normal, $\frac{4}{5}$, or the homozygous quintuple recessive, "5." The same process can then be repeated in every generation, by crossing the normal-appearing sons to their recessive sisters. It is evident that the "5" females which are used need not be virgin, as they could have been fertilized only by $\frac{4}{5}$ or by 5 males. When $\frac{4}{5}$ males are crossed by 5 females which have been made up so as to contain in addition the dominant factor streak (these 5 females need only be heterozygous for streak; homozygous streaks are hard to handle), the daughters

[4] From Bridges.

[5] From Morgan.

which appear streaked but otherwise normal, must have the composition: $\frac{d_a\ j \quad c_v \quad b_a}{S_{tr}\ b_1\ p_u\ v_g\ a_r\ s_p}$. These are the "$F_1$" females whose gametic output is to be tested. Accordingly, they are crossed to normal males. All the offspring ("F_2") appear normal (except for the dominant, streak), but the factors they received from their mother may be determined by mating them, individually, to ♂♂ males, for the latter contain (heterozygous) all recessive characters possible in the former.

It was at first thought that labor might be saved, and certain points in addition determined, by conducting the linkage determinations on flies heterozygous for the factors used in both chromosomes I and II at the same time, instead of making determinations of the linkage in the two chromosomes in separate experiments. The multiple stocks of the two chromosomes were, therefore, crossed together, and females were finally obtained that had the composition:

$$\frac{y\ w\ A\ b\ v\ m\ s\ r\ f}{cc_1 \qquad B_r} \qquad \frac{S_{tr}\ b_1\ p_u\ v_g\ a_r\ s_p}{d_a\ j \quad . \quad c_v \quad b_a}$$

These females, heterozygous for 22 mutant factors, were then crossed to normal males, and the composition of their female offspring was tested by mating these in separate bottles to ♂♂ males. The maintenance of the double-multiple stocks proved to be extremely difficult, however, and so, after obtaining determinations for 166 offspring from such females, the two groups of mutant factors were again separated. The data obtained in this part of the experiment show that there is no linkage of any of the twelve factors studied in group I with any of the ten studied in group II; this is of course in marked contrast to the relations shown between factors in the same group. The conclusions of previous workers that no factor in one group was linked with any factor in another group were based on results obtained with comparatively few combinations of factors, which were chosen as samples, so to speak. It will be seen that in the present work these conclusions have been confirmed by a study of 132 differ-

ent combinations of factors in group I with factors in group II.

CLASSIFICATION OF FACTOR COMBINATIONS TRANSMITTED BY FEMALES HAVING THE COMPOSITION: $\frac{y\ w\ A\ b\ v\ m\ s\ r\ f}{c \quad c_1 \quad B_r}$

	Yellows		Grays		Totals
	Non-cross-overs				
	ywAbvmsrf	186	*c c₁ B_r*	200	386
Between	*Single Cross-overs*				
y and w	*yc c₁ B_r*	2	*wAbvmsrf*	5	7
w and A	*yw c₁ B_r*	3	*cAbvmsrf*	5	8
A and b	*ywA c₁ B_r*	4	*c bvmsrf*	11	15
b and c_1	*ywAb c₁ B_r*	17	*c vmsrf*	27	44
c_1 and v	*ywAb B_r*	46	*c c₁ vmsrf*	51	97
v and m	*ywAbv B_r*	7	*c c₁ msrf*	9	16
m and s	*ywAbvm B_r*	18	*c c₁ srf*	19	37
s and r	*ywAbvms B_r*	28	*c c₁ rf*	38	66
r and f	*ywAbvmsr B_r*	0	*c c₁ f*	5	5
f and B_r	*ywAbvmsrf B_r*	0	*c c₁*	1	1
Between	*Double Cross-overs*				
y and w; c_1 and v	*yc c₁vmsrf*	1	.....		1
y and w; m and s	.....		*wAbvm B_r*	1	1
y and w; s and r	*yc c₁ rf*	1	*wAbvms B_r*	1	2
y and w; r and f	*yc c₁ f*	1	.....		1
w and A; c_1 and v	*yw c₁vmsrf*	1	.....		1
w and A; r and f	*yw c₁ f*	1	.....		1
A and b; c_1 and v	.....		*c b B_r*	1	1
A and b; s and r	*ywA c₁ rf*	1	.....		1
b and c_1; m and s	*ywAbc₁ srf*	1	*c vm B_r*	1	2
b and c_1; s and r	*ywAbc₁ rf*	4	*c vms B_r*	3	7
c_1 and v; v and m	.....		*c c₁ v B_r*	1	1
c_1 and v; s and r	*ywAb rf*	7	*c c₁ vms B_r*	1	8
c_1 and v; r and f	*ywAb f*	2	.....		2
c_1 and v; f and B	*ywAb*	1	.....		1

Total Double and Single Crossing-over

Between	Observed Number	Per Cent. of Crossing-over	Between	Observed Number	Per Cent. of Crossing-over
y and w	12	2	v and m	17	2
w and A	10	1.5	m and s	40	6
A and b	17	2	s and r	84	11.5
b and c_1	52	7.5	r and f	9	1.2
c_1 and v	112	16.	f and B_r	2	0.3

Not only the independence of the factors in the two groups was shown by this experiment in which the two groups were followed at once, but also the independence of the crossings-over. In the total of 166 cases, there were 81 in which chromosome I underwent crossing-over (either

single or double), and 101 in which chromosome II crossed over. If it was a matter of pure chance whether or not crossings-over occurred in I and II at the same time, coincident crossing-over should have happened in $\frac{81}{166} \times 101 = 49+$ cases. The actual number of cases in which crossing-over occurred in both chromosomes at once was 52. Thus there is neither interference nor synchronism of these crossings-over, and this result too is strikingly dissimilar to the relations found between two crossings-over in the same chromosome.

Since the results in the two chromosomes were found to be independent in all respects, it is deemed unnecessary to list here all the different combinations which were found of factors in group I with factors in group II, and their observed frequencies. The results for the two chromosomes may more advantageously be separated and added to the other results, obtained when groups I and II were followed in different crosses.

The data for the first chromosome are given in the tables which follow. In all, 712 offspring of females heterozygous for the 12 mutant factors in group I have been tested.

The above results give a direct demonstration of the fact that the factors behave as though they are joined in a chain; when interchange takes place, the factors stick together in sections according to their place in line and are not interchanged singly. The fact is shown so patently as to require no further comment.

Non-crossing-over occurs in this chromosome in 54.4 per cent. of cases, single crossing-over in 41.7 per cent., double crossing-over in 4.2 per cent. No triple crossing-over was obtained in this count, although one, which will be described later, was obtained in the next generation, in one of the "testing out" bottles.

(To be concluded)

SHORTER ARTICLES AND DISCUSSION

THE CAUSE OF THE BELIEF IN USE INHERITANCE

THIS note expresses an effort to view the old and recurring problem of use inheritance from the aspect of the underlying motives of thought involved instead of through a consideration of the evidence directly bearing upon it.

The heredity of acquired traits is, theoretically, biological heresy. But the interminable cropping out of the belief even in professional circles indicates a strong psychological impulse toward the conviction. The mainspring of this impulse thus becomes a matter of some importance to the student of heredity.

To begin with, it is well known that the lay public almost without exception takes use inheritance for granted. Even evolution, in the real mental workings of most educated but unprofessional people, is more generally explained, unconsciously and in concrete cases, by appeal to the machinery of use inheritance than to that of selection. The phrases struggle for existence and survival of the fittest have indeed evoked a wide popular response on account of their picturesqueness, but their concepts are still but little employed, even in the intelligent and studied folk mind, as a real means of understanding or explaining evolution.

Those sporadic but in the aggregate numerous biologists who adhere to the doctrine of use inheritance, revert to it, or evince symptoms of a leaning toward it, may be divided into two types. The first class, probably because they think more penetratingly than the average, long ago perceived the inadequacy of selection alone as an explanation of organic evolution; and more lately perceive also the insufficiency of selection with mutations and Mendelian phenomena superadded. To students of this type, use inheritance is therefore merely a last resort, a hypothesis on which they fall back in default of any other to stop a logical gap. The only methodological criticism that can be made of this school is that it would undoubtedly be more stimulating of new discovery if we were frankly to avow the limits of our knowledge and leave certain things unexplained, than to complete the mental structure of evolution by piecing in a principle which admittedly rests only on contested facts and has opposed to it about as large a body of evidence as can be assembled on behalf of any negative and therefore logically unprovable proposition.

The second class consists of biologists and utilizers of biological material whose keenness of thought is below the average. This school introduces use inheritance into the conception of evolution because it has failed to comprehend adequately the essential problems of evolution, and approaches them substantially in the attitude of the layman.

The latter class is therefore merely unscientific and popular in its thought processes; the former, having exhausted scientific means and found them inadequate, returns, more or less frankly in despair, to current folk opinion. The problem accordingly is to discover the basis of the deeply rooted popular notion that is involved in both cases.

While never formulated into a definite working principle until Lamarck, because of the world's lack of specific scientific interest in organic phenomena, the principle of use inheritance has nevertheless been tacitly assumed by civilized nations of all periods, and is taken as self-evident even by savages. It must therefore rest on a large mass of common experience interpreted by an elementary process of thought. Such an elementary process—in fact the only elementary process of wide scope—is analogy.

The question then becomes what may be the basis—real enough though unscientifically employed—for the analogizing that has resulted in the conviction that use heredity exists. There must evidently be a broad group of phenomena in human experience that bear some resemblance to the hereditary transmission of the acquired.

These phenomena are the exceedingly common ones of social inheritance or cultural transmission and growth. We do "inherit" a name, or property, or knowledge of a language, or the practice of an art, or belief in a particular form of religion. Biologically such "inheritance" is of course absolutely distinct from "heredity" because the mechanism of transmission is different. The source of social inheritance is not restricted to parents and actual ancestors in the line of descent, but embraces a multitude of individuals, consanguineous and unrelated, dead, living, and sometimes even junior to the inheritors; in other words, the totality of the social environment, past and present, of an individual. We can and do "inherit" property from an uncle, our "mother tongue" from a nurse, the arithmetic evolved by past ages from a schoolmaster, our dogmas and philosophy from a prophet, our political and moral beliefs from the whole circumambient public opinion.

As this social or cultural transmission concerns human beings, it is of more immediate interest to the normal unschooled mind than the transmission which gives organs, instincts and peculiarities to animals and plants. It is therefore recognized much sooner than the processes which guide biological or organic transmission. It needs no proof that in his development man was concerned far earlier with himself than with animals or other parts of nature. It is well known, for instance, that the animism which is accepted as the basis of all religion, anthropomorphizes not only its gods and the vaguer forces of nature, but especially animals, plants and objects.

It is only recently, accordingly, that the world has paid any true attention to organic heredity, whereas since the beginning of human existence there has been recognition of social inheritance. History, the science of human society, is, even in a relatively advanced form, several thousand years old, and as a rudiment has enough interest to appeal to savages. Biology, the science of the organic, has an age of barely two centuries.

It is significant that the first theory of organic evolution, that of Lamarck, resorted wholly to the explanation of use inheritance borrowed from social inheritance. A second stage was reached when Darwin introduced the organic factor of selection, though refusing to break with the older explanation. A last phase was inaugurated when Weismann insisted that organic phenomena must be interpreted solely by organic processes.

The priority of reasoning by analogy over reasoning by means of a specific mechanism is a world-wide historical phenomenon. The two modern views of evolution and creation are found as crude cosmic philosophies in the mythologies of the most primitive savages, as well as in the thinking of Hindus, Semites, Greeks, and Romans. But they occur, one as an analogy with the familiar phenomenon of manufacture or making of objects by hand, the other as an analogy with the equally familiar phenomena of birth and growth. What modern science has done is to adopt these age-old and crude ideas, as it has adopted the half-mythologic concepts of the atom and ether, and put them to new use. Only the uneducated think of Darwin as the originator of the doctrine of evolution. What he originated was an organic and in his day new mechanism, by which the old concept of evolution could be explained and therefore supported.

The distinction between the social and the organic is far from

a novel one. But the two groups of phenomena, and the processes involved in each, are still very frequently confounded in other domains than that of use inheritance. The whole eugenics movement, for instance, so far as it is a constructive program and not a mere matter of ordinary practical prophylactic social hygiene, rests upon the assumption that social progress can best be accomplished by organic means. It may be rash to deny wholly that such an end can be achieved in this way or that it would be useful. But the orthodox eugenist, from the time of the founder Galton, has consistently and complacently made this assumption without any inquiry as to its justification. Lamarck erected a false doctrine of evolution through explaining the organic in terms of the social, or in terms derived by mere analogy from the social. The eugenists of to-day, it may fairly be suggested, bid fair to vitiate a movement that springs from the most sincere of motives, by resting its basis on an interpretation of the social as merely organic.

In summary, the doctrine of the hereditary transmission of acquired characters is no more disprovable than it is provable by accumulation and analysis of evidence. It springs from a naïve, unscientific, and even primitive method of reasoning by analogy, which in this case works to a confusion of the long-distinguished and necessarily distinct concepts of the organic and the social. The doctrine must therefore be dismissed on purely methodological grounds. It is possible that when the missing factor or element of evolution is discovered that neither Darwin nor the mutationists have been able to find, this factor will prove to be something superficially similar to use inheritance. But it will differ from the present only half-discredited but discreditable factor of heredity by acquirement, in containing an organic mechanism, and will therefore be essentially different from this crude and confused assumption.

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TRIFOLIUM PRATENSE QUINQUEFOLIUM

HUGO DE VRIES in his mutation theory tells us in detail about his production, by means of selection from two mutant forms, of a five-leaved race of red clover. This race he called *Trifolium pratense quinquefolium*. The two plants obtained for starting

his selection were found, according to the author, growing near the edge of a road that was covered with grass. He does not tell us the exact composition of all the leaves of these two plants with which he started, but states that they bore several tetramerous and one pentamerous leaf. Neither of the plants, therefore, could be called mutants of a new race, but were mutating forms from which De Vries obtained, after a process of most rigid selection, his highly variable race, *Trifolium pratense quinquefolium*.

During the spring of 1914, I found growing in an old orchard at Corvallis, Oregon, a red clover plant that showed "full-fledged" all the characters of *Trifolium pratense quinquefolium* about which De Vries has written, and which took him so long to obtain by the aid of selection. This clover plant, after careful examination, was transplanted in one of my experimental plots for further study. The following descriptive notes of it are given: Of medium height; good color; normal as to vigor, but not luxuriant; seven stalks; leaves, 4 trimerous, 5 tetramerous, 12 pentamerous; total number of leaves, 21. Not only did the pentamerous condition of so many leaves represent the mode for leaf variation, but there were more five-leaved leaves than both four-leaved and three-leaved leaves combined.

The magnitude of this mutation may be more fully appreciated when we reflect that De Vries, after selecting for three generations and obtaining 300 plants, found only one that gave as high a percentage as 36 for both tetra- and pentamerous leaves; while the percentage of tetra- and pentamerous leaves for all those counted, 8,366, was only 14.

After finding this specimen of *Trifolium pratense quinquefolium* I was exceedingly desirous of obtaining another plant with which to cross fertilize it so as to obtain a race which could be used commercially, but repeated searches made for many days failed to reveal any other plant suitable to cross with this one. Thus failing to find a second plant, I decided to propagate the discovered mutant vegetatively. This method gave some degree of success, and a few plants were reared during the summer of 1914 from slips. When I left Oregon at the end of the summer, four of these plants were transferred to a private lot, and a railing, supported by stakes, was put around them.

Examination on June 3 the following summer (1915) showed two of these slips doing well, one had been trampled on and

killed by a cow, and the other was dead. At this time I still had hopes of obtaining a race of five-leaved red clover, but when I returned to Oregon and examined these plants on June 20 all such hopes vanished, for a neighbor's cow had completely ruined them, cropping off all the stalks down to the ground. None of the plants revived after this last injury.

Records for the leaf production of this mutant were kept, and from them I have obtained the following: On May 11, 1914, a count was made of all the leaves produced up to date. There had been produced 6 trimerous, 7 tetramerous and 17 pentamerous leaves; 30 in all, over 56 per cent. pentamerous. We notice, however, a slight decline in the percentage of pentamerous leaves produced, since the plant was found. This decline, early noticed, continued throughout the summer, and on August 23, when I made my last leaf counts, I obtained the following record of leaf production for this mutant clover plant:

	Trimerous Leaves	Tetramerous Leaves	Pentamerous Leaves
Leaves on plant when found	4	5	12
Leaves produced since plant was found	30	11	17
Total leaves produced by plant	34	16	29

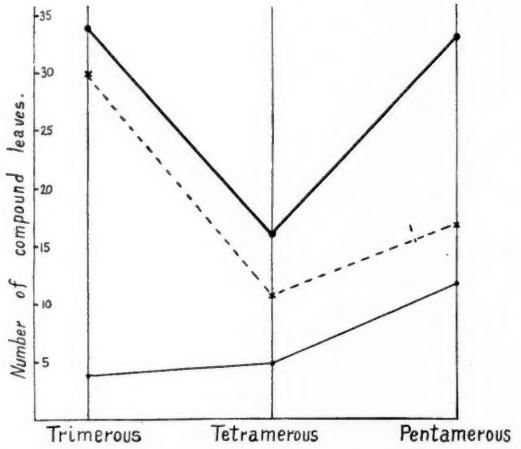

FIG. 1. Frequency polygon showing leaf variations of a mutant individual of *Trifolium pratense quinquefolium*. Light solid line shows variations of leaves on plant when discovered; dotted line, variations in leaves produced by plant in captivity; heavy solid line, variations for all leaves produced by mutant plant and its slip plant descendants.

How are we to interpret these results? Why should there be such a preponderance of pentamerous leaves produced during the early growth period of the plant, then a preponderance of trimerous leaves during the latter part of the season?

The records obtained for 1915 for the slip plants added but little to the 1914 records. The leaf production of slip plant No. 3, however, is interesting. In 1914 this slip plant produced 4 trimerous, 2 tetramerous and 4 pentamerous leaves. In 1915, however, it produced 0 trimerous, 0 tetramerous and 4 pentamerous leaves—showing strongly the inherited tendency to produce the pentamerous leaves during the second season. Clover heads were produced during the summer of 1914, but no seed was found in them.

No leaves were produced by this plant having more than five leaflets, a condition that obtained during the first three generations of De Vries's race, yet later he obtained both 6- and 7-merous leaves in abundance. A frequency polygon is plotted (see figure) for the leaf variations of this red clover mutant.

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NOTES AND LITERATURE

FAUNAL DISPERSAL[1]

THE widely different conclusions drawn by different students from the same facts is well illustrated by Matthew's recent paper on the geographic distribution of animals.[2] Although these differences are of many and varied sorts a fundamental one seems to be concerned with the question as to whether the peripheral part or the central part of the range of a group contains the more progressive members of that group.

The idea which is held, either consciously or unconsciously, by many is that after a group has arisen it spreads; and the migrants, meeting new conditions, develop new characters. These new forms then spread still further and develop other characters. Thus the more primitive members remain at or near the point of origin of the group; the successively more progressive members will be found at respectively greater distances from this point. According to this theory we may trace *backward* the dispersal of the group by following the distributions of the several members from the most progressive to the most primitive. We may not be able to follow this line to the actual point of origin of the group for the most primitive members may not be alive now and we may be unable to find their fossil remains. However, as organisms usually extend their range in more than one direction, we may be able to trace several lines of dispersal and deduce that the point of origin was near the place at which these lines tend to converge.

The other theory is as nearly the opposite as can be. According to it the first progressive makes its appearance well within the range of the primitive members of the group. If the new form is not an improvement over the primitive one, it dies out. If it be an improvement, it crowds the primitive form which is forced to leave its place of origin and migrate. It is sometimes made a part of this theory (but it is not a necessary part) that the reason a new form appeared among the old was that the new climatic conditions appeared there and that if the old form could, in its migration, keep in climatic conditions suited to it, well and good; if it could not, it either died out or became adapted to the

[1] Read at the February 15, 1916, meeting of the N. Y. Entomological Society.

[2] W. D. Matthew, 1915, "Climate and Evolution," *Annals*, N. Y. Acad. of Sciences, XXIV, pp. 171–318.

climatic conditions into which it was forced without, however, losing all the earmarks of its primitiveness. Later still, newer forms appeared at the old stand and forced their predecessors still further away. Therefore, if we follow the distribution of the several members of the group from the most progressive to the most primitive, we will trace the dispersal of the group *forward,* not backward.

It would probably be impossible to decide, either by logomachic methods or by watching present-day movements of species, which of these two theories is correct. However, we are not left without hope because paleontologists are daily digging up evidence which, when sufficiently complete and properly translated, will leave us in no doubt as to the history of the dispersal of certain groups, and there is little doubt that the same general principles which hold for those groups will apply to others concerning which we have not and probably never will have fossil record. Of course details may differ from group to group, but it is not probable that there is one set of laws for mammals and another for reptiles, one for birds and another for insects, that nature constructed a trans-oceanic bridge for one group, but stationed a guardian angel on it to prevent the passage of others. The chief differences probably were such as the differing responses of the different groups to changes of the environment and the differing powers of different groups in overcoming given barriers to dispersal.

Next to that brought about by the holding of the diametrically opposing theories just discussed, perhaps the most important source of confusion is in not keeping clearly distinct "center of origin," "center of dispersal" and "center of greatest development." They may all be in the same region or they may be as far removed from each other as the earth's surface will permit but they are not the same. A group may arise at *A* and move to *B,* from which point there are easy paths of migration to *C, D, E* and *F*. It may, and doubtless would, go to all of these but it might find that *E* alone furnishes good conditions for its future development. Its center of origin would then be at *A,* its center of dispersal would be at *B,* and its center of greatest development at *E*. According to the first theory discussed above, the more primitive forms would be found at *A* or *B* and the later developments at *C, D, E* and *F,* but chiefly at *E*. According to the second theory, *C, D, E* and *F* would have the primitive forms (although they might be much modified, especially at *E*) and the progressive members of the group would be at *A* or *B*.

Adams[3] did good service in bringing together a number of suggested "criteria for the determination of centers of dispersal." It was not to be expected, and doubtless Adams did not expect, that all of them would stand up under a test. Without attempting to exhaust the subject, present anything new or review all that has been said about them, certain notes may be made in connection with this discussion, taking up the criteria *seriatim*.

1. *Location of the Greatest Differentiation of the Type.*—I believe it is more than a mere question of definition to say that the offering of this criterion is an instance of the confusion of "center of dispersal" with "center of greatest development." Two of the stock illustrations of great differentiation are those of marsupials in Australia and lemurs in Madagascar. Since these groups are so greatly developed on the respective islands they should, according to this criterion, have spread out from these islands to the rest of the world. Unless the paleontological evidence, as brought together and interpreted by Matthew, is false, that was not the history of these cases. On the other hand, wherever their points of origin were, their ancestors got into the Holarctic region and then spread in various directions. Now, if this be true, their center of dispersal would be Holaretica, although their greatest differentiation *at the present time* is toward the other end of the world. Lest the quibble should be raised by others, it should be stated that the real fundamental center of dispersal of a group is its center of origin and that there are

[3] C. C. Adams, 1902, "Southeastern United States as a Center of Distribution of Flora and Fauna," *Biol. Bull.*, VII, p. 122. After the present paper had gone to the press I obtained through the kindness of Professor Adams, a portion of "An Ecological Survey of Isle Royale, Lake Superior" prepared under his direction and published (1909) as a part of the Report of [Michigan] Board of the Geological Survey for 1908. In this he takes up, again, these criteria. He says "It should be clearly emphasized that it is the convergence of evidence from many criteria which must be the final test in the determination of origins rather than the dependence upon any supposedly absolute criterion." He discusses the various criteria in greater detail than was done in his 1902 paper and adds, as another criterion, "Direction indicated by seasonal appearance; vernal suggesting boreal or montane origin and aestival as austral or lowland derivation." I regret that I did not have earlier access to this paper but as I did not base my present remarks on his 1902 contribution with the idea of criticizing it in particular but for the purpose of pointing out the danger of relying too confidently on such evidence, it is probably unnecessary to change the text of this article in order to meet the expanded discussion in the 1909 paper by Adams. This is especially true since I intentionally omitted, for the sake of brevity, reference to certain papers, such as the one by Tower (1906), which bear on the same subject.

other centers of dispersal wherever there are branches in the forward movement of the group; but I understand the usual meaning of "center of dispersal" to be the point or points from which the principal lines went out which resulted in the wide spread of the group.

2. *Location of Dominance or Great Abundance of Individuals.*—This criterion seems futile. A group may have moved in all directions from a given region and died out entirely in the region from which it moved. Nothing is more clearly established than the changeableness of geologic climate and hence of all environmental factors and we know of many instances where nothing is left of a group of organisms but fossil remains in the regions of their former abundance and a few living remnants in far-away, protected spots. If, as seems very clear, we can not believe that even the most populous of these havens marks the center of dispersal of such a group, neither can we apply this criterion with safety to any other group. The area of present dominance is merely that area, of all those now inhabited by a group, which is at the present time most suited to the group—unless, of course, it has arrived in a more suitable area so recently that it has not had time to develop its dominance.

3. *Location of Synthetic or Closely Related Forms.*—This criterion will be considered more in detail later, but it may be remarked in passing that the location of closely related forms is of little help in arriving at the center of dispersal unless we know whether these forms are more primitive or the reverse and unless, furthermore, we have selected the right one of the two opposing theories which were mentioned in the beginning of this discussion.

4. *Location of Maximum Size of Individuals.*—It is difficult to see why individuals should be larger at the center of dispersal than elsewhere. Possibly they may be larger the nearer they are to present-day, optimum environmental conditions, but this is probably not often, and certainly not necessarily, anywhere near the ancient center of dispersal.

5. *Location of Greatest Productiveness and its Relative Stability, in Crops.*—Adam says this criterion is very closely related to the second one. If so, it fails for the same reason. Also, it would seem that the latter part of this criterion conflicts with criterion number one—"greatest differentiation of type."

6. *Continuity and Convergence of Lines of Dispersal.*—This certainly ought to work, provided we follow the lines in the right direction. The difficulty is that north and south lines on a more

or less spherical world converge either way we go. Furthermore, if we place confidence in oceanic bridges which have been washed away, piers and all, the lines of dispersal are apt to be frequently discontinuous.

7. *Location of Least Dependence upon a Restricted Habitat.*—For example, certain plants and animals which, in the region of New York City, are found only in sphagnum bogs, such as those of the Jersey pine barrens, occur more widely distributed farther north. According to this criterion their center of dispersal would be in the north and this may be true. Again, lizards occur, in the region of New York City, only or chiefly in these same pine barrens, while farther south they run about wherever they can get sunshine. According to this criterion the ancient center of dispersal of lizards was somewhere near the equator, but this may not be true.

8. *Continuity and Directness of Individual Variations or Modifications Radiating from the Center of Origin along the Highways of Dispersal.*—This criterion is probably stated rather more fully than was meant. If we knew all this, the problem would be solved. Perhaps Adams meant that continuity and directness of modifications ("individual variations" gets us into the biometric-mutation discussion and that is another story) point out the highways of dispersal from the point of origin. His reference on the next page to Osborn's law of adaptive radiation indicates that this is the proper interpretation of this criterion and, if so, the present author has no quarrel with it except to point out once more that we are left in doubt as to which way to follow the lines.

9. *Direction indicated by Biogeographical Affinities.*—I am not certain as to what this means. I suppose a given group which is neotropical at the present time has biogeographical affinities with other present-day neotropical groups. If we know the centers of dispersal of the other groups we have a working hypothesis concerning the center of dispersal of the group in question. If this be what is meant, it seems to be probable.

10. *Direction indicated by the Annual Migration Routes, in Birds.*—This criterion is meant to apply only to birds and I fear we know too little concerning the intricate problems of bird migration to say whether their present-day routes of annual migration follow the route of ancestral dispersal or not. Probably they do not, as the birds would be expected to change their routes with changing environmental conditions. Furthermore, although it is believed that birds return to their ancestral home

to breed, this is not so firmly established as to leave no doubt about which is going and which is coming.

Adams clearly stated that these criteria are for use where "we do not have paleontological evidence in sufficient abundance to materially aid us," but I confess to a feeling that we must still depend on paleontology to give us the general laws of dispersal. It is for this reason that such papers as Matthew's seem to me so helpful. I regret that the organisms in which I am most interested did not leave more marks on the sands of time, but if the great majority of mammalian groups left records to show that they followed a certain set of lines of dispersal and the end result is of a given character, it seems worth while to compare the end results of the dispersal of other forms with those of the mammalian groups. If the comparison is close, the deduction that the lines of dispersal also are comparable seems not unsafe. It certainly seems unwise to construct trans-oceanic bridges where conservative geologists say there could have been none, especially if they must be made so tenuous that only insects, spiders, snails, earthworms, fresh-water fishes and such small fry can cross, mammals being forbidden.

Several of the criteria given by Adams which appear to fail in helping us discover the ancient centers of dispersal seem to be indicators of present or potential centers. Thus the first one: a region where there is a "great differentiation of type" within a group would seem to be a region prepared to send members of that group into all the world. If paths exist or chance intervenes, this group should be able to fit some, at least, of its many different types into the new environments which it encounters in its spread. If all the surrounding territory is already occupied by more successful competitors, that merely means that there are no paths for the dispersal of this group. One of the real, but sometimes overlooked, barriers to faunal spread is the presence of competitors. This, however, does not negate the idea that a region of "great differentiation of type" is a potential center of dispersal.

In somewhat the same way the region of "dominance or great abundance of individuals" is a potential center of dispersal. The case is not so clear; but it would seem that where there is a *relatively* large supply of individuals more could be spared for, or would be forced into, colonization efforts than where there are relatively few.

This suggests that what was the center of greatest development (especially in variety of types) became the center of dis-

persal, and that what is now the center of greatest development is or will be a new center of dispersal provided there are means of dispersal. There seems to be a genetic relationship between these three centers and, of course, at the beginning the center of origin is also the other two.

Little need be said here about means of dispersal except to point out what Matthew also emphasized, namely, that time is long and luck is real. Those of us who have been brought up on the doctrine of evolution by selection of "chance" variations should have a whole-hearted respect for that sometimes abused word. Those who believe there has been time enough for alert Nature to seize upon enough chances to differentiate 400,000 species of insects, for example, need not strain unduly in swallowing the notion that a very small proportion of these have been able to get across relatively short stretches of water without a bridge. Those who believe that Nature not only seized upon but made opportunities for the differentiation of species should have no trouble in discovering easier ways for her to help her creatures spread their range than by raising up long narrow portions of the ocean bed for a certain few to cross dry shod and then sinking it to the discomfiture of those which are not of the elect.

Insects probably get about as easily as any creatures because most insects fly or may be blown long distances and, furthermore, the majority have at least two distinct stages in their life cycle during which they remain inert and without the necessity of feeding. If mammals can reach islands not connected by bridges, surely insects of many kinds can. Matthew places great stress on natural rafts as a means of transporting mammalian fauna. After giving briefly a "series of facts and assumptions [which] may serve to give some idea of the degree of probability that attaches to the hypothesis of over-sea transportation to account for the population of oceanic islands" he says:

> If then we allow that ten such cases of natural rafts far out at sea have been reported, we may concede that 1,000 have probably occurred in three centuries and 30,000,000 during the Cenozoic. Of these rafts, only 3,000,000 will have had living mammals upon them, of these only 30,000 will have reached land, and in only 300 of these cases will the species have established a foothold.[4] This is quite sufficient to cover the dozen or two cases of mammalia on the larger oceanic islands.

Few of these assumptions can be statistically verified. Yet I think that, on the whole, they do not overstate the probabilities in each case.

[4] Matthew apparently changed, between pages 206 and 207, his ideas concerning the probabilities. However, it is the general notion and not the actual figures which is important.

They are intended only as a rough index of the degree of probability that attaches to the method, and to show that the populating of the oceanic islands through over-sea transportation, especially upon natural rafts, is not an explanation to be set aside as too unlikely for consideration.

I confess to some haziness as to the probabilities here set forth, but, if they are anywhere near true, entomologists need not worry. In addition to their creatures not needing rafts as badly as do mammals, it is certainly probable that every, not "one in a hundred," natural raft big enough to be noticed and recorded by voyagers contained not one, but many, insects. Smaller rafts or even single trees might contain many individuals of several species and, since a single fertilized female gives birth to many offspring, the chance of a given species establishing itself on virgin soil is much greater than it is in the case of mammals. Furthermore, insects have been dispersing since before the Carboniferous. Many of the islands may not be that old, but this simply means that insects have had a chance at such an island since the first wavelets rippled about its uplifting peak. The one thing which may be comparatively disadvantageous to insects is that many of them are rather closely bound up in their food relations with certain plants, but this disadvantage is somewhat decreased by the fact that, if phytophagous insects are carried on natural rafts, their food plant is likely to be a part of the material which makes up the raft and both may be established together. The pros and cons are numerous and involved. It is a balancing of probabilities with the burden of proof on the side which claims the right to make over major features of the earth's surface in the face of contrary geological evidence.

If this be true for the scant fauna of oceanic islands, what shall we say of the suggested bridges, running this way and that, across the oceans for the purpose of connecting continental faunas and floras, especially in equatorial regions? Mercator gave us a map of the world so constructed that the longitudinal lines are parallel from the north pole to the south. Now the fact is that a degree of longitude equals approximately 111,300 meters at the equator, 104,600 meters at 20° latitude, 85,400 meters at 40°, 55,800 meters at 60°, 19,400 meters at 80°, and no meters at the poles. Therefore the equatorial distances on Mercator's projection are relatively far too short. On the globe or on a proportional projection in which a meter at the equator is as long as a meter in Alaska we see that north of the Tropic of Cancer in the eastern hemisphere lies a huge land mass consist-

ing of Europe, northern Africa and most of Asia. This mass almost touches at its northeastern corner the somewhat smaller mass of North America. The intervening space, Bering Strait, is only about sixty-five miles wide, very shallow, and dotted with islands. The other gap between these masses is somewhat wider, but still not so great as the shortest distance between South America and Africa. Thus the arctic region is almost encircled by land and itself contains much land. The earth's surface is really one huge northern land mass with three southward projections, namely America, Africa and the East Indian Islands, including Australia. Antarctica is a small disconnected mass at the other end. A species or group of species originating in, or getting into, the far north could, as far as the present configuration of the continents goes, populate the earth and have solid ground under its feet most of the way.

Probably one of the main reasons for the little consideration which students of geographic distribution have given to this route of dispersal is the present climatic conditions in the far north. It seems to have been easier to imagine the ocean's bottom heaved up between Africa and South America than to conceive of a different climate in the northern regions. Yet we have definite and incontrovertible evidence of mild arctic climate in at least several geologic periods, while the only moderately strong evidence of a bridge across the Atlantic, for example, is the presence of related or identical forms on the opposite shores.

Suppose two of us are known to have been together on Broadway, but now one of us is in eastern Connecticut and the other at the eastern end of Long Island. One theory might be that we traveled together to the eastern end of Connecticut and then, while one of us stayed there, the other crossed the Sound in some way or other, even though we could not swim that far, there was no regular boat service, and the only evidence of a bridge having been built and then destroyed is that one of us is in eastern Connecticut and the other in eastern Long Island. It would seem to me more probable that we parted company in Manhattan and, while one of us crossed by a known bridge to Long Island and then had good going along the southern shore of the Sound, the other crossed known bridges and travelled through Connecticut along the northern shore of the Sound. This seems to be the way the respective theories concerning the biogeographical relationships of South America, Africa and Australia stand except that we have some other facts. To continue the comparison: although there may be no direct evidence as how one of us came to be in

eastern Connecticut and the other at the eastern end of Long Island, yet it is known that other people have left New York and gotten to these two places without crossing the eastern end of the Sound and, furthermore (to make the comparison more accurate), we must put in as a part of the argument that no one was ever actually known to cross directly from eastern Connecticut to Long Island or *vice versa*. In the face of such evidence he would be rash indeed who would hold to the first theory as to our movements after we left Broadway.

Now I know very little about geology and still less about fossil mammals, but I am willing to take on faith the conclusions of well-accredited students of these subjects if the conclusions seem to have been reached by logical deduction from reasonable premises and if the assertions of fact are not too widely different from my recollection of the assertions of fact made by students who have reached other conclusions from what seem to me to be unreasonable premises. It would be out of place here to give the details of Matthew's analysis of vertebrate, especially mammalian, paleontology. He takes up group after group and outlines their fossil record showing that they

> accord fully and in detail with the principles[5] here set forth, and to be impossible of explanation except upon the theory of permanence of the ocean basins during the Cenozoic era. While the prominence of the Holarctic region as a center of dispersal is ascribed to its central position and the greater area, some evidence is given to show that climate is also a factor in the greater progressiveness of the northern, since it is also noticeable in the southern as compared with tropical faunæ.
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> The distribution of the Reptilia appears to be in conformity with the principles here outlined, and extends their application to the Mesozoic era. The distribution of birds and fishes and of invertebrates and plants is probably in accord with the same general principles, modified by differences in methods of dispersal. The opposing conclusions that have been drawn from the distribution of these groups are believed to be due to an incorrect interpretation of the evidence. A few instances, which have been prominently used to support opposing conclusions, are analyzed and shown to conform to the conclusions above set forth, if interpreted upon similar lines as the data for mammalian distribution.

As an example of the widely divergent conclusions which may be drawn from the same data concerning present-day distribution

[5] Permanency of the abyssal oceans; climatic cycles from extremes of cold or arid zonar climates culminating in glacial epochs, to the extremes of warm, humid, uniform climates, associated with cycles of moderate elevation and submergence, respectively, of continents; the cyclic development of progressive groups of animals in the great northern land mass and their dispersal southward as the result of these climatic changes.

I take the liberty of referring to my paper on the spiders of the Greater Antilles.[6] On pages 139 and 140 it is shown that sixteen Antillean genera, selected in a rather random fashion, are found to-day in regions connected by a certain hypothetical system of trans-oceanic bridges and not elsewhere. In some cases there appears to be almost no specific difference, even, between spiders whose present range is far from continuous. In fact, this set of data and other instances mentioned in that paper seem to me to furnish as strong evidence for such bridges as is given by the recent distribution of any one group of organisms, and yet I felt that this was not at all the explanation. It seemed more reasonable to believe that spiders had dispersed by the way of Holarctica on land masses which were practically the same as they are now and that the present discontinuous distribution of the ancient types is brought about by the fact that they are merely relicts which are now found far separated from each other.

Entomologists will at once think of a number of species which have reached the United States in historic times from Mexico and many will use these in a contention that much of our fauna has been derived from the south. I believe that the movement has been largely the other way and that the "southern element" of our fauna is largely made up of those things which have dropped behind in the general southern movement. As in any stream there are back eddies, so in the stream of dispersal we must expect back eddies (especially when man makes a channel as he did when he planted a large number of potatoes up to the former habitat of the Colorado potato beetle or grew great quantities of corn and other cereals for the chinch bug) but the eddies do not indicate the direction of the main stream. In their progress toward the equatorial region the streams of dispersal leave pools here and there—the stranded relicts of an ancient fauna. There are doubtless numerous swirls and back currents, while near their mouths these streams of dispersal may be much subject to "tides" due to minor, *i. e.*, measured by centuries, fluctuations of climate, but their general movement is, nevertheless, from the poles toward the equator. Since the northern polar regions have the larger land masses and better facilities for such dispersal-stream flow, the larger movements have been from the north, but simliar, though smaller, currents are to be expected in the southern hemisphere.

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[6] Lutz, F. E., 1915. "List of Greater Antillean Spiders with Notes on their Distribution." *Annals*, N. Y. Acad. Sciences, XXVI, pp. 71–148.